

## **Historic, Archive Document**

Do not assume content reflects current scientific knowledge, policies, or practices.



280,39

*Do not list in B of A*

15  
9.2

A Summary of Current Program 7/1/67  
and Preliminary Report of Progress  
for 7/1/66 to 6/30/67

\*\*-\*

EASTERN UTILIZATION RESEARCH AND  
DEVELOPMENT DIVISION

U. S. DEPT. OF AGRICULTURE  
NATIONAL AGRICULTURAL LIBRARY

of the

OCT 23 1967

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

CURRENT SERIAL RECORDS

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1966, and June 30, 1967. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

July 1, 1967



## TABLE OF CONTENTS

	Page
Introduction.....	iii
Area No. 1, Dairy Utilization -- Food.....	1
Area No. 2, Meat Utilization -- Food.....	22
Area No. 3, Animal Fats and Oils -- Industrial Products..	32
Area No. 4, Hides and Leather Utilization.....	44
Area No. 5, Potato Utilization -- Food.....	55
Area No. 6, Vegetable Utilization -- Food.....	60
Area No. 7, Deciduous Fruit and Tree Nut Utilization -- Food.....	63
Area No. 8, Tobacco Utilization -- Industrial Products...	67
Area No. 9, Maple Sap and Sirup Utilization -- Food.....	74
Line Project Check List.....	78



## INTRODUCTION

The mission of the Eastern Utilization Research and Development Division is to develop new and expanded markets for designated farm commodities, through research to develop new and improved products and processes based on these commodities. The Division conducts research on dairy products, meat, animal fats, hides, tobacco, maple sirup and Eastern fruits and vegetables including potatoes.

In carrying out its mission, the Division does research in physical and biological science and in engineering throughout the spectrum of basic research, applied research and pilot plant development. Division scientists are aware of the key role that basic research plays in uncovering new information that may be later exploited in applied research and development. Hence, a substantial portion of the Division's effort is in basic research. When appropriate, engineers conduct pilot plant studies of promising laboratory developments to provide engineering and cost data essential to industrial application feasibility determinations.

The research effort at the Eastern Division amounts to approximately 178 scientist man-years. In addition, the Division supervises domestic research contracts and grants equivalent to 41.6 scientist man-years per year at 33 locations in the United States. The Division's program is supplemented by a variety of research projects in foreign countries under PL-480 grants. There are now a total of 37 such grants in 12 countries.

The Division is organized in 10 laboratories of which 5 are located entirely at the Eastern Regional Research Laboratory, Wyndmoor, Pa., one is at Beltsville, Md., with part of its research program at Wyndmoor, and one is at Washington, D. C., with part of its research program at Beltsville. One of the laboratories at Wyndmoor has some of its research located at East Grand Forks, Minnesota, and a second has research located at Lexington, Kentucky. One of the Division's laboratories is devoted to pioneering research on the biophysical mechanisms of macromolecules, and is located at Brandeis University, Waltham, Massachusetts.

In every phase of their research, Division scientists cooperate with representatives of colleges and universities, state experiment stations, research institutes and associations, industrial organizations and with other Government agencies. Much of the cooperation is informal, but some work is conducted under conditions described in written cooperative agreements and memorandums of understanding. Currently 9 such agreements are in effect.

The farm products with which the Eastern Division deals provide more than half of the nation's cash farm receipts; the major part of U. S. farmland suitable for cultivation is used to provide feed for livestock and dairy cattle; in seven states tobacco provides more cash receipts than any other field crop. It is thus evident that maintaining and enlarging the markets

for these and the other farm products under study at the Eastern Division should be a major national concern.

Division scientists have already achieved much both in terms of discoveries now commercialized and discoveries of a fundamental nature that will be exploited in the future. Following are some examples of recent developments based on research at the Eastern Division and on Division-supervised research that typify such achievements.

#### Examples of Recent Accomplishments of the Eastern Utilization Research and Development Division

Eastern Peach Processing Industry Expands. Millions of trees of the new clingstone peach variety "Babygold" have been planted recently in Pennsylvania, New York, Ohio, Virginia, Illinois, Michigan, the Carolinas, Arkansas and Canada. The superior processing quality of this peach was discovered as a result of USDA contract research at the New Jersey Agricultural Experiment Station. A screening of the Station's extensive collection of fruit varieties revealed the commercial value of Babygold. These peaches rate high for making baby food puree, hence their name, and for canning as halves and slices. Eastern growers especially are enthusiastic about the Babygold variety for it promises to make them independent of the fresh market as the sole outlet for their harvest. Arkansas, however, is credited with the first commercial crop--several hundred tons in 1966.

Ultraviolet Radiation Preserves Apple Cider. The shelf-life of a perishable farm product, apple cider, can be extended by exposure to germ-killing ultraviolet radiation. This treatment followed by refrigeration preserves the cider as effectively as chemical additives. In addition, the radiation causes no detectable flavor change. Using inexpensive small-scale equipment producers can now make full-flavored cider with a shelf-life adequate for distribution from road-side stands, dairy cases or dairy routes.

Commercial-scale Gun Designed for Explosive Puffing of Fruits and Vegetables. Commercial use of the USDA-developed explosive puffing process for making dehydrated fruits and vegetables is being expedited by the design of a puffing gun for use with a wide variety of fruits and vegetables. The gun employs both injected superheated steam and external heat; residence time in the gun is shortened and output is increased nearly four-fold over earlier models. Pilot plant studies have found it suitable for use (in multiples) for large-scale operations. A local manufacturer has fabricated several for commercial use. The Department engineers who designed the gun have made detailed construction drawings available to industry and have published directions for its installation and operation, together with a detailed cost estimate on quick-cooking carrot and potato dice. The former are presently manufactured commercially by explosive puffing.

Continuous Process for Intensifying Maple Sirup Flavor. A continuous high-flavoring process developed by ARS scientists has been received with enthusiasm by the two principal packers who use more than 70 percent of the



available bulk maple sirup in their cane-maple blended products. The process is based on a high-temperature short-time treatment in which the liquid sirup is pumped through heat exchangers under high pressure. At temperatures 70° to 100°F. above that used in making maple sirup, flavor precursors interact rapidly and more completely to yield a 2- to 40-fold increase in flavor. The operator can also control the color and flavor intensification for specific applications.

With this process, 80 to 90 percent of the U. S. commercial sirup production can be made to meet the blenders' color and flavor specifications. In poor crop years, such as 1967, these high-flavored sirups help extend the crop to supply sufficient sap for the blending segment of the industry.

Macromolecules in Cigarette Smoke. In Department studies on the relationship between cigarette smoking and health, an unusual and complex material--a pigment of very high molecular weight--has been isolated from cigarette smoke. The pigment contains iron, chlorogenic acid, at least 18 amino acids, a silicone material, and several alkaloids, including nicotine. The pigment is the first reported instance of the presence in tobacco smoke of nicotine or other alkaloids in a component of high molecular weight. There is evidence that the smoke pigment comes from a pigment in the tobacco leaf.

Cigar Filler Types Characterized. Four types of cigar filler tobaccos--Pennsylvania, Columbian, Puerto Rican and Dominican--have been distinguished from each other objectively through analysis of the tobacco smoke by gas chromatography. Each smoke displayed unique--and hence characteristic--patterns on the chromatograms. Easiest to identify this way was Columbian smoke, which also showed the most characteristic flavor and odor. The research was cooperative with the Cigar Manufacturers Association.

Heat-treated Milk Fat for Food Use. The flavor of many foods may be enhanced through the use of heat-treated milk fat. It imparts a desirable flavor ranging from buttery and butterscotch to caramel--depending on the treatment--to the food to which it is added. This distinctive improved flavor is pleasing to consumers.

In the process, sweet cream is pasteurized, cooled and churned into butter, which is subsequently heated to 220°F. to evaporate water. The remaining mixture of milk fat, protein, lactose and milk salts is heated to 230°F. to 280°F., depending on the flavor intensity desired. Then the nonfat milk solids are removed.

The product, which is 99.8 percent milk fat, maintains its flavor during storage, even at room temperature. It has been successfully tested in candy, ice cream and ice milk, and in cooking meats, fish and other foods. As a flavor source, less of the heat-treated milk fat is needed than butter, which has long been used to flavor foods.

Commercial Use of Foam-Spray Drying for Skim Milk. Foam-spray drying, a technique in which air or some other gas at high pressure is injected into the liquid-feed line of a spray dryer, has now been applied to the commercial-scale manufacture of nonfat dry milk. In the dryer the gas liquid-feed mixture makes tiny droplets of foam, which dry as expanded particles. These make an "instant" powder--one which quickly dissolves when water is added to it. The new technique provides substantial savings in both capital and operating costs compared to the conventional equipment used to make instant powder. The foam-spray dried skim milk is used in food manufacture and for direct consumer use. Commercial interest is growing and additional applications of the process are anticipated.

Oxidized Flavor in Milk Averted by Improved Feed Practices. ARS-supported research at the University of Maryland has demonstrated that oxidized flavor in milk--a defect associated with winter and dry-lot feeding of dairy cows--may be averted by supplementing the dairy ration with vitamin E. The vitamin may be added as a concentrate by the feed manufacturer. The vitamin E naturally present in most green forages may be preserved by ensiling or by rapid dehydration of freshly harvested forage. The information developed by the research scientists will permit dairy farmers to provide for adequate vitamin E by proper selection and harvesting of forage for silage. The research findings will be applicable to dry-lot dairy operations and to the manufacture of dehydrated alfalfa and other forages.

Industrial Chemicals from Surplus Fats. Department scientists have perfected several chemical reactions which yield a variety of industrially valuable aromatic compounds from the oleic acid in surplus tallow. For example, phenylstearic acid is a viscous oil which remains liquid over a wide temperature range (-50 to +300°C.). It is a good lubricant and surface active agent. The properties of another compound, hydroxyphenylstearic acid, make it useful in lubricants, antioxidants, plasticizers and resin intermediates. Two large chemical corporations have developed industrial processes for making these materials. Phenylstearic acid is expected to sell at 28-33 cents a pound, and hydroxyphenylstearic acid at 35-40 cents a pound. Within a few years, millions of pounds of phenylstearic acid will find use in the lubricant field alone. If easily available at low cost, these compounds should provide an important new outlet for surplus inedible fats.

Improved Glove Leather. As a result of USDA leather tanning research, golfers will soon be able to buy leather gloves that will last all season or longer under hard usage. The gloves will remain soft and comfortable throughout their useful life even though they are wet many times with perspiration and washed repeatedly with soap and water. Without doubt, similar gloves for other sports, such as handball, will follow.

The ARS-developed tannage is applicable to both dress and work gloves and has attracted the attention of many tanners and glove manufacturers. The industry appreciates the value of this research, for substantial amounts of leather go into glovemaking. In 1965, for example, the USA produced and imported well over 25 million dozen pairs of leather and part-leather gloves.

Leather Defect Traced to Inherent Weakness in Hides. Cooperative research between ARS and the University of Cincinnati has established that "pulpy butt" leather results from hide defects and not from poor preservation or improper processing practices. The afflicted hides were shown to possess an abnormal fiber orientation, especially in the back region, which weakens the leather for shoemaking. The fibers are arranged in a loose and vertical pattern rather than a compact and horizontal weave, as seen in normal hides. These characteristic fiber structures were observed in at least 10% of the plumper Hereford steer hides and to a lesser extent in the heavier Angus hides examined. As a consequence of this research, the tanning industry may be able to prevent an annual \$5 to \$10 million loss. It is presently working on means for detecting the defective leathers and removing them from shoemaking channels.

Comfort Properties of Shoe Leather Determined. Indian scientists working under a PL-480 research grant have reported on the properties of leather which contribute to foot health, comfort and wearability. With continued use, the unique three-dimensional protein fiber network of leather was shown to increase in flexibility; it conformed to the foot with increasing humidity; it became more permeable to both air and water vapor. Leather was found to absorb an appreciable quantity of perspiration, thereby lowering the humidity within the shoe and making it more comfortable. As yet, these properties have not been duplicated in any of the man-made substitutes. Continued research to detect and measure the unique characteristics of leather will be helpful in defining and objectively measuring its comfort properties. Properly advertised and promoted, this information should give shoe leather a competitive advantage over the newly-developed synthetic substitutes.

Identification of Meat Smoking Constituents. Out of over 200 compounds which have been reported to be present in wood smoke, ARS scientists discovered that only a few phenolic constituents are needed to impart a highly acceptable smoked flavor to meat products. In this work, the wood smoke was analyzed and separated into many fractions so that the flavoring potential of each could be judged. As this research continues, additional data will be obtained and applied to increasing the effectiveness, the reproducibility and the safety of meat smoking processes.

Color Development in Sausage Making. In recent basic studies on the chemical reactions involved in meat curing, Department scientists have obtained practical information regarding the successful development of stable cured color in sausages. Modern methods of sausage production allow only a minimum time for color formation. Processors, therefore, must add reducing chemicals to accelerate the reaction between nitrite and the natural meat pigment. In these investigations, the rates of reaction for cured meat color formation were determined with various reducing chemicals under sausage-making conditions. The resulting data should prove valuable to meat processors using computer-programmed sausage formulations. It will allow them to choose the best reductant and the best set of conditions for any of several practical situations.



The Eastern Division is one of four research divisions of the Agricultural Research Service conducting utilization research and development. The other Divisions are the Northern at Peoria, Illinois, the Southern at New Orleans, Louisiana, and the Western at Albany, California. It is evident from the examples cited above that Utilization Research can make highly valuable contributions to agriculture.

As a step toward implementation of the recommendations for a National Program of Research for Agriculture made jointly by the Association of State Universities and Land Grant Colleges and the USDA, a section has been added to this introduction and to each of the Areas in this report.

#### Examples of Recent Accomplishments of the State Agricultural Experiment Stations

Water Conservation in Potato Processing. Water usage and management in potato processing plant operations was studied by Maine food scientists. Factors found to affect water use and reuse were: volume of raw materials processed per day; conditions of raw materials at time of processing; loadings of total suspended solids and microbiological populations in effluent waters from various processing steps; and wide variations in processing procedures within individual plants. Consideration for reuse of waters in potato processing plant operations requires evaluation on a continuing basis of total suspended solids, certain soluble solids, microbiological populations, their types, and effect on the finished product as well as other factors. Consideration of reuse of water will also require constant examination to insure that quality and safety of the finished product are in no manner adversely affected.

Natural Peach Flavorings Best For Use in Ice Cream. Research at the Georgia Agricultural Experiment Station shows that peach varieties differ in their ability to flavor ice cream. Natural peach flavorings, pureed or dried, were superior to artificial flavorings in ice cream. Peach puree or dried peach flakes used at 5.0 - 7.5% make a good quality ice cream which can be stored for 2 months without significant changes in flavor quality. Peach purees are preferred over dried peach flakes because they impart a fresh peach flavor to ice cream, whereas the dried product possesses a slight "preserved" flavor.

Color Appeal in Vegetables. Research at the North Carolina Station shows the changes chlorophyll undergoes during processing and storage of green vegetables. At least 12 chlorophyll derivatives have been extracted from processed green vegetables. This research points out the importance of proper storage temperature for color maintenance, and the effect of certain metallic salts in color change. Salts of copper or zinc present in trace amounts in ingredients used for pickle manufacture will intensify undesirable color. Equipment used for processing vegetables may be the source of trace amounts of metals which will cause an unnatural color in the processed product.

New "Instant Fruit." Consumer products have become available as the result from a process of freeze-drying crushed fruit into pellet form developed by North Carolina Station food scientists. These pellets provide the homemaker with the same "instant" convenience in fruit that she enjoys in such products as coffee, potatoes, and milk. Several food firms are now testing the peach, strawberry and blueberry pellets in prepared mixes for cakes, pancakes, muffins and other products. In addition, this process provides an outlet for overripe fruit.

Meat Packaged in Edible Film. University of Illinois food scientists have developed an edible film for packaging meat that minimizes bacterial growth, moisture loss, and pigment loss. The film is prepared from sodium alginate, crystalline corn syrup solids, and calcium propionate. In comparative studies at 30°C. with rib-eye steaks, control samples were spoiled after 6 days whereas the samples dipped in the film preparation did not spoil until after 10-12 days. Dipped samples retained red pigment for 10-12 days, but the control samples were a dark brown color after 7 days. Samples were judged to have acceptable odor, flavor, and texture after storing 10 days at 30°C.

Rapid Ham Processing. Recent research at Oklahoma State University, cooperative with USDA, shows that ham can be satisfactorily processed within 15 hours from time of slaughter. The shorter processing time is accomplished by eliminating the 24-hour chill treatment. Hams were alternately assigned to rapid or conventional processing. Bacterial counts and pH changes were determined as they were influenced by processing techniques. As a result of the rapid processing, curing, pH, smoking, and cooking there was a reduction in associative vegetative bacteria. This research indicates that major bacteriological problems do not exist in the accelerated processing of hams.

Sterile Tissue For Meats Research. Sterile tissue techniques are being used by researchers at the Ohio Agricultural Research and Development Center to remove germ-free samples of tissue from a carcass. This technique provides samples that may be stored at room temperature for extended periods. These unprocessed samples provide excellent control material for microbiological studies to determine the microflora that affect the flavor and quality of meat. Basic information from this research could provide leads to improving color retention, flavor, and shelf life of meats.

Antibiotic Activity of Lactobacilli. As a result of the claimed therapeutic value of acidophilus milk, researchers at the Nebraska Agricultural Experiment Station investigated the antibacterial activity of strains of Lactobacillus acidophilus. One strain was shown to be inhibitory toward gram-positive and gram-negative organisms. Milk was found to be the best media for producing maximum antibacterial activity. Maximum activity occurred at 37°C. with an incubation period of 48 hours. The antibiotic, tentatively named "Acidophilin," has been partially purified and found to be active against several bacterial pathogens and nontoxic to rats.



AREA NO. 1. DAIRY UTILIZATION - FOOD

Problem. Utilization research on milk is conducted in order that the milk products industry can defend and expand its markets by (1) developing new and improved products and (2) by developing new and more efficient processing techniques.

The importance of this research can be gauged from the vast size of the industry, for dairying is one of the largest segments of American agriculture, milk is the base of an enormous processing and distributing systems, and milk is nutritionally important:

Farm cash receipts from milk and cream marketings provide more than five billion dollars a year, about 13% of all cash receipts from farm products; only livestock marketings are greater.

The retail value of milk and milk products is eleven billion dollars a year.

Milk production is over sixty million tons a year; dairying is first in farm income in 9 states, second in 6, third in 7 and important in all the rest.

The farmer's share of the consumer's food dollar spent on dairy products averages 44 cents whereas the average share for all foods is about 38 cents.

Milk contributes about a quarter of the protein in the American diet and most of the calcium, phosphorus and vitamin B<sub>2</sub>.

Per capita consumption of milk in the United States has been decreasing steadily and is now about 579 pounds per year, well below that of many foreign nations, including Finland, Ireland, New Zealand, Canada, Australia, Sweden, Denmark, Norway, Switzerland, Belgium and the United Kingdom, all of which consume more than 800 pounds per capita. It is thus evident that there is opportunity to increase milk consumption, despite the tremendous variety of ways in which our populace can spend its money, and the freedom it has in deciding what or what not to buy.

Milk is a biological secretion and a valuable human food, but basically it is a complex mixture of complex chemical substances. Finding out what these substances are, what their individual properties are, how to speed up, slow down, direct and, in sum, control their reactions is vital to practical development of new markets based on new products or processes, improvement in existing products processes, or cost reduction in existing processes.

The lead time to such development may be several years. This kind of research is recognized as a province of government laboratories, state and national,



since it is basic to the whole milk industry and the results are freely available to all. Outside public research, such studies could be undertaken only by the very largest industrial laboratories and these would, quite understandably, disseminate results only as they saw fit and doubtless for their own profitability.

Government properly sponsors only research and development that the milk industry can not be expected to do for itself: projects of long duration requiring larger resources, having a substantially greater element of risk or, as with basic research, lacking the prospect of full private exploitation.

Government research and development on processes and products normally proceeds to such a technical stage that industry can logically decide whether or not to adopt them and, when adopted, government may properly also provide technical advice during the first stages of commercial application.

#### USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, microbiologists, food technologists and engineers engaged in basic research on the composition and properties of milk and in applied research directed to the development of new and improved dairy products and processing technology. The Department's research facilities are located in Wyndmoor, Pa., Washington, D. C., and Beltsville, Md. In addition, some Division research work is conducted at Brandeis University, Waltham, Mass.

The Federal (USDA) scientific effort devoted to research on milk totals 61.9 scientist man-years which includes 6.6 scientist man-years in the domestic contract and grant research program. This effort is distributed as follows:

(a) Chemical Composition and Physical Properties. Research at Wyndmoor, Washington, and Waltham, totals 25.9 scientist man-years, devoted to properties of protein complexes in milk, properties of the various caseins in milk, the interaction of milk proteins in solution, composition and structure of protein components of milk, the behavior of milk enzymes, and study of ribosomal nucleic acids.

Contract research at the University of Maryland, College Park, (0.5 scientist man-year) deals with the relation of milk fat composition to the diet of the cow. Contract research at the University of Minnesota, St. Paul, (0.5 scientist man-year) is concerned with the possible role of genetics in affecting the heat stability of individual milks. Contract research at Ohio State University, Columbus, (0.6 scientist man-year) deals with the calcium phosphate complex in milk and milk concentrates. In addition, research sponsored by the Department under PL-480 grants is in progress at the following foreign institutions:



1. Indian Institute of Science, Bangalore, India, on phosphoproteins of milk (5 years, 1963-1968).
2. National Dairy Research Institute, Karnal, Punjab, India, on the proteose-peptone fraction of milk (5 years, 1963-1968).
3. Israel Institute of Technology, Haifa, on the formation of unnatural nucleic acids (3 years, 1965-1968).
4. University of Uppsala, Uppsala, Sweden, on the development of methods for purification of milk proteins and studies of their structure (5 years, 1963-1968).
5. College of Agriculture in Olsztyn, Poland, on mechanisms of the cheese-ripening process (5 years, 1963-1968).
6. University of Graz, Graz, Austria, on the structures and interactions of nucleic acids by means of small angle X-ray studies (5 years, 1966-1971).

During the year, research under PL-480 grants was completed at (a) Institut National de la Recherche Agronomique, Paris, France, on the non-protein nitrogenous substances formed from milk proteins during industrial treatments, (b) Centre de Recherches sur les Macromolecular, Strasbourg, France, on the subunit structure of nucleic acids, and (c) Instituto Nacional de Tecnologia, Rio de Janeiro, Brazil, on the structure and properties of proteolytic enzymes.

(b) Flavor. Research at Washington involves 9.9 scientist man-years devoted to the study of stale flavor and flavor stability in sterile milks. In addition, grant research at the University of Maryland (0.5 scientist man-year) is devoted to milk flavors and flavor precursors that are derived from pasture or dry feeding practices. Grant research at Oregon State University, Corvallis, (1.0 scientist man-year) is directed to isolation and identification of specific flavor contributing compounds in butter. Grant research at the Pennsylvania State University, University Park, (0.9 scientist man-year) is devoted to study of the origin and control of lactones, methyl ketones and their precursors in milk as a basis for the development of procedures to avoid the undesirable flavor effects of these substances in milk products.

In addition, research sponsored by the Department under PL-480 grants is in progress at the following foreign institutions:

1. National Dairy Research Institute, Karnal, Punjab, India on sulfur compounds in milk

and milk products and their relation to cooked flavors and oxidative stability (5 years, 1963-1968).

2. Biochemical Institute, Helsinki, Finland, on dietary factors controlling flavor in milk (5 years, 1964-1969).

(c) Color, Texture and Other Quality Factors. Research at Washington totals 3.0 scientist man-years devoted to investigation of the allergens of cow's milk and of the physical stability of sterilized milk. Contract research equivalent to 1.5 scientist man-years is in progress at the Pet Milk Company, Greenville, Illinois, to study the stability of commercial fluid milk during refrigerated storage over periods great enough to permit the radioactivity of iodine-131 to decay to harmless levels.

Research was initiated under a PL-480 grant at Hebrew University, Hadassah Medical School, Jerusalem, Israel, to study the immunological reaction of infants to cow's milk (3 years, 1966-1969).

(d) Microbiology and Toxicology. Research at Washington involves 1.0 scientist man-years devoted to study of the chemical and physical mechanism of the development and maintenance of heat resistance and dormancy in bacterial spores. Contract research at the University of Wisconsin, Madison, (0.1 scientist man-year) concerned with the effects of nonfat dry milk on bread yeast fermentation was completed. In addition, research under a PL-480 grant is in progress at the U. P. Agricultural University, Pantnagar, India, on factors which influence the synthesis of dipicolinic acid in bacterial spores (5 years, 1966-1971).

(e) Technology - Process and Product Development. Research on process and product development totals 15.5 scientist man-years at Washington, Wyndmoor and Beltsville, on the preparation of dry whole milk by the vacuum foam-drying process (Wyndmoor), the foam spray-drying process (Washington), the development of improved dairy processing equipment, new product development based on butter fat, and investigation of survival of Salmonella during cheese manufacture. Grant research at North Carolina State University, Raleigh (0.7 scientist man-year) deals with physical changes in milks due to steam injection. Heat transfer in powdered milk will be studied under a grant at Michigan State University, East Lansing, (0.3 scientist man-year). Contract research on removal of radionuclides from milk by fixed- and moving-resin-bed systems was completed.

Additional research sponsored by the Department under PL-480 grants is in progress at the following institutions:

1. National Dairy Research Institute, Karnal, Punjab, India, on the isolation and use of milk coagulating enzymes for cheese manufacture (5 years, 1962-1967).

2. National Dairy Research Institute, Karnal, Punjab, India, on the role of starter bacteria and some genetic variants in the development of flavor during the manufacture of cheese (5 years, 1966-1971).
3. "Juan de la Cierva" Foundation for Applied Research, Madrid, Spain, on the thermal and related physical properties of milk and milk products (5 years, 1964-1969).
4. Technical University Berlin, Berlin, West Germany, on chemical changes at the surface of fat globules in foam-dried whole milk (4 years, 1963-1967).

During the year, research at Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, was completed on the addition of nonfat dry milk solids to buffalo milk in the manufacture of hard cheese.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 67.6 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition and Physical Properties

In normal  $\beta$ -lactoglobulin polypeptide linear zigzag and  $\alpha$ -helical forms predominate. The zigzag or pleated sheet structure is concentrated near the molecule surface. Controlled changes in the proportion of ordered versus disordered parts of the molecule and in the type of order can be produced by altering the solvent.  $\alpha_{s1}$ -casein B, the most frequently occurring genetic variant of the calcium-sensitive casein of cow's milk, has a molecular weight of 24,600 in 3 M guanidine hydrochloride at pH 7 as determined by sedimentation equilibrium. In glycine-NaOH buffer (pH 12,  $I = 0.5$ ,  $25^\circ$ ) it degrades slowly and irreversibly and after one week molecular weight is 14,000. For the three major caseins of cow's milk,  $\alpha_{s1}$ ,  $\beta$  and  $\kappa$ , a very low  $\alpha$ -helix content and a low degree of structural organization is evident. However, appreciable  $\alpha$ -helix formation can be produced by dissolving the casein in a solvent such as 2-chloroethanol or acidic methanol.

Stable bacterial ribosomal nucleic acid (H-RNA) was prepared by carefully freeing the material from traces of ribonuclease. Molecular weight measurements at ionic strength of 0.15 and 0.01 confirmed that this H-RNA is a mixture of two components with molecular weights of 1,000,000 and 500,000. Degradation experiments indicate that either ribosomal RNA is a closed loop or has cross-links between segments of the polynucleotide chains. Hence, the usually accepted structure of a long random chain (mostly single stranded) must be rejected.

In research at the Israel Institute of Technology, Haifa, the formation of "unnatural" nucleic acids was studied in two bacterial systems, one infected by virus particles (T4). Upon infection of a bacterial cell by a virus, the only new ribonucleic acid (RNA) formed is messenger RNA.

The work on the isolation and characterization of acid ribonuclease, acid phosphomonoesterase and exonuclease, conducted at the Centre de Recherches sur les Macromolécules, Strasbourg, France, was completed. The methods of preparation were derived from that devised for acid DNase. The preparation of these enzymes is significant because, (1) the enzymes were obtained as highly purified preparations during the chromatographic purification of acid DNase; (2) acid phosphomonoesterase and spleen exonuclease were essential for determining the specificity of acid DNase, (3) acid RNase, acid phosphomonoesterase and acid DNase are the first enzymes from lysosomes prepared in a homogeneous state.

The terminal oligonucleotides obtained by digesting DNA with acid DNase were studied in detail. Their average size was equal to 10 residues 30-35% of the digest being tetra- to octanucleotides. The remaining 65-70% were larger oligonucleotides.

At the University of Graz, Graz, Austria, research is continuing on the study of the structures of nucleic acids by means of small angle X-rays. By plotting the cross-section factor, pure DNA produced the expected radius of gyration ( $R = 8.4\text{\AA}$ ) and the known mass per unit length. The addition of actinomycin effected an increase in intensity at small angles. This is explained as a cross-linking effect.

In research cooperative with EU, Dr. John Gregory of Rockefeller University found that one molecule of milk ribonuclease B contains one molecule of galactosamine and apparently 2.6 molecules of glucosamine confirming EU results obtained by less elegant methods. Whether the fractional number of glucosamines is due to imprecision of method or accurately indicates the average content of an inhomogeneous protein is not known. The acetyl-, propionyl-, isobutyryl-, and butyryl-derivatives of  $\beta$ -casein A' have been prepared by reaction of the acid anhydride in a dimethylsulfoxide solution. At least 90% of the ninhydrin-positive material in the protein is lost, indicating that the amino groups are acylated to this degree. Dimethylsulfoxide is a particularly good solvent for acylating the caseins for both casein and acid anhydrides are soluble in this reagent.

In enzyme research at EU, milk ribonuclease B and pancreatic ribonuclease B were separated by column chromatography, thus confirming differences observed earlier for carbohydrate composition.

$\kappa$ -Casein (kappa-casein) preparations from individual cow milks show either one or two major components (probably genetic variants) and several minor components all part of the  $\kappa$ -casein complex as judged by ability to stabilize  $\alpha_s$ -casein and to be acted on by rennin. In this research at EU it was found that the action of rennin leads to formation of homogeneous carbohydrate-free



p(ara)- $\kappa$ -casein and macropeptides, some of which contain carbohydrate. The insoluble p- $\kappa$ -casein may be coupled to bovine serum albumin to prepare soluble derivatives. These derivatives can form stable complexes with  $\alpha_{s1}$ -casein in the presence of calcium ion, suggesting that the association between  $\alpha_{s1}$ -casein and  $\kappa$ -casein is a property of the p- $\kappa$ -portion of the molecule. The inability of  $\kappa$ -casein to stabilize  $\alpha_s$ -casein when photooxidized suggests that the site of association may involve histidine and perhaps tryptophan of  $\kappa$ -casein. When the calcium sensitive components of  $\alpha_s$ - and  $\beta$ -caseins were modified by reaction with formaldehyde or cyanate ion, the calcium-binding capacity of these proteins was not changed but the modified proteins no longer precipitated in the presence of calcium ion. Furthermore,  $\kappa$ -casein similarly modified by a formaldehyde or cyanate ion no longer stabilized  $\alpha_s$ -casein toward calcium ion. Amino acid analysis indicates that lysine residues are implicated. Crystalline polymorphs of  $\beta$ -lactoglobulin have been treated with sodium dodecyl sulfate-S35 and the derivatives were crystallized and analyzed by scintillation counting methods. All contained 2 moles of bound dodecyl sulfate per mole of protein.

Adsorbents capable of selectively binding particular proteins are being developed at the University of Uppsala, Uppsala, Sweden.

At the Pioneering Research Laboratory for Physical Biochemistry, Waltham, Mass., research is continuing on (a) interactions between proteins and solvent components and (b) conformational changes in  $\beta$ -lactoglobulin. It has been found that chloroethanol (strong structure former) is bound preferentially to  $\beta$ -lactoglobulin below 40% (v/v in water), whereas above this concentration water is bound preferentially.

Contract research at the University of Minnesota with 3:1 concentrated skim milk demonstrated no consistent difference in the pH-heat stability of milks containing  $\kappa$ -casein A and  $\kappa$ -casein B. Thus, variations in heat stability of milks have not correlated with  $\alpha_s$ ,  $\beta$ , or  $\kappa$ -casein types. The heat stability of mixtures of pairs of individual milk samples from casein-typed cows was not necessarily the mean of the two individual samples even though dialysis supposedly eliminated salt effects. Evidently there are complex interactions here that are not understood.

In research conducted at Wyndmoor, amino acid analysis of a newly isolated  $\beta$ -casein polymorph designated A<sup>3</sup> was found to contain 4 residues of histidine per molecule compared with 6 in  $\beta$ -casein A<sup>1</sup>, 5 in A<sup>2</sup>, and 6 in  $\beta$ -caseins B and C. A second  $\beta$ -casein polymorph, designated D, was prepared from the milk of a zebu cow. It differs in content of lysine, histidine, arginine and alanine from  $\beta$ -casein B of the zebu cow. "Difference peptides" from  $\alpha_{s1}$ -caseins B and C were isolated; the extra glycine residue which distinguishes  $\alpha_{s1}$ -casein C from B is found in the difference peptide of C while the extra glutamic acid residue characteristic of  $\alpha_{s1}$ -casein B is in the difference peptide of B. The two peptides are otherwise identical.  $\gamma$ -Casein occurs in at least two different forms, designated A and B, as shown by disc gel electrophoresis. The polymorphism is probably genetically controlled.

In comparative studies of  $\beta$ -casein from cow and buffalo milk, it has been found that, as in cow milk, polymorphism of this component of casein occurs in buffalo milk as well. An apparatus was devised for electrophoretic separation of the polymorphs on acrylamide gel. This research is in progress at Indian Institute of Science, Bangalore, India.

Basic research on the mechanisms of the cheese-ripening process was continued at the College of Agriculture in Olsztyn, Poland. Most attention was given to the identification and characterization of products formed on the decomposition of casein by rennin. These include peptides, amino acids and other degradation products. Model systems were used to study extracellular protease of Streptococcus lactis which is actively proteolytic. Rennin split off glycopeptide from  $\kappa$ -casein causing coagulation. It also continued an active proteolytic role during cheese ripening.

Electron microscope studies at EU on the size distribution of casein micelles showed a slight increase in micelle size with decrease in temperature from 26°C. to 0°C. Centrifuged skim milk showed no separation of micelles by size. The casein micelles of a 3:1 sterilized skim milk concentrate and a similar concentration with added polyphosphate, examined weekly for four months by electron microscope techniques showed no difference in micelles between milks with and without polyphosphate although gelation occurred in the control sample. The micelles of the concentrated milks were about 2- to 2-½ times as large as micelles from fresh single strength skim milk. In grant research at Ohio State University, Columbus, the nuclear magnetic resonance studies of pure casein components and salt solutions was extended to milk systems, and electron microscope studies of the micelles in the milk samples undergoing nuclear magnetic resonance studies have also been initiated.

In PL-480 sponsored research at the National Dairy Research Institute, Punjab, India, the composition and size of the proteose-peptone fraction of cow and buffalo milk was found to be influenced by genetic makeup of the milk producer, stage of lactation and heat treatment. Paper electrophoresis, gel filtration and a new colorimetric procedure developed on this project were used to study the observed variance in the proteose-peptone fractions. These methods were also used to follow the changes in the proteose-peptone fraction of milk exposed to the action of rennet. A further study of the proteose-peptones in various dairy products showed measurable differences between these fractions in evaporated milks made in India and the United States.

Contract research at the Maryland Agricultural Experiment Station showed that cows receiving corn silage as their only forage produced slightly more milk than those receiving both hay and corn, and their milk was higher in fat, protein and solids-not-fat. The stage of lactation appears to have some effect on fatty acid composition. In early lactation the percent of oleic acid was unusually high and palmitic acid unusually low compared with late lactation. These changes appear to be due to a more rapid decrease in oleic acid than to total fatty acid content at later stages of lactation.

Differential thermal analysis (DTA) and calorimetry are now being used in a study at EU of properties of milk fat. Heats of melting and specific heats of milk fat and DTA studies on the crystallization behavior of a hydrogenated milk fat have been carried out.

The volatile carbonyl and hydroxy compounds in normal and in "zero" milk fat were determined. Zero milk is obtained from cows on a purified, protein-free diet. It is thus free from flavor substances possibly carried over from conventional feeds. The results of the determinations are as follows: (a) an array of volatile carbonyl compounds exist in both fats; (b) a new class of carbonyl compounds exists in both milk fats; (c) both normal and zero milk fat contain the C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>7</sub>, C<sub>9</sub>, C<sub>11</sub> and C<sub>13</sub> methyl ketones although some of these may be generated during distillation; (d) both normal and zero milk fat contain a series of saturated aldehydes from C<sub>3</sub> through about C<sub>14</sub>; (e) both normal and zero milk fat contains a series of 2-enals from C<sub>5</sub> through about C<sub>12</sub>; neither milk fat contains any detectable 2,4-dienal. (Cooperative with Professor A. Virtanen, Biochemical Institute, Helsinki, Finland).

#### B. Flavor

Sterile fluid milks containing added kynurenine and tryptophan were prepared; after three months' storage at 80°F. no significant flavor difference between the supplemented and control samples were observed. The O-aminoacetophenone believed to be a major contributor to the off-flavors appears to be associated with the whey proteins. The observation that steam deodorization of butteroil yields a butteroil product stable for more than a year when stored under nitrogen at 80°F. but that sterile milks prepared with steam deodorized butteroil developed a stale flavor, suggests that the skim milk portion of the milk is a major contributor to the stale flavor in sterile milk.

A number of aromatic compounds have been identified by the application of gas chromatographic and mass spectrometric techniques to the volatiles obtained from low temperature steam distillation or steam stripping of cream butter serum and butteroil in grant research at Oregon State University. The origin of these compounds is unknown, but literature reports suggest that they may originate in animal feeds. Preliminary taste panel studies suggest that certain lactones, methyl ketones, fatty acids, aldehydes and dimethylsulfoxide contribute significantly to the flavor of butter. Grant research at the Pennsylvania State University indicated that metabolic disturbances such as ketosis and parturition dramatically decreased the concentration of lactone potential of milk fat. A pronounced seasonal trend was observed, lactone concentration being higher in winter than in summer milk. Since it was established earlier that feeding practice can affect lactone potential, it seems clear that lactone precursors are apparently by-products or end-products of endogenous metabolism.

During the year research at the National Dairy Research Institute, Punjab, India, under a PL-480 grant showed that the sulfhydryl content of both buffalo and cow's milk varied seasonally, being high in September and February and low in May. This may reflect stage of lactation and feed of the producers since



both were found to influence the sulfhydryl content of milk. When cow's milk was stored at 0 - 4°C. sulfhydryl content dropped more rapidly in buffalo milk. Preliminary results indicate sulfhydryl compounds in milk activate alkaline phosphatase and may reactivate the enzyme activity lost during heat treatment. Work on the isolation and characterization of the low molecular weight sulfhydryl containing compounds in heated milk has been initiated.

The study of dietary factors controlling flavor in milk is continuing under a PL-480 grant at the Biochemical Institute, Helsinki, Finland.

Milk production of test animals maintained on a protein-free diet continues to increase and an annual yield of 9000 pounds of milk has been achieved. Analysis of the various constituents of milk continue to emphasize the close similarity between test and normal milk.

Grant research at the University of Maryland demonstrated that supplementing a basic ration of alfalfa hay and grain with  $\alpha$ -tocopherol acetate eliminated the susceptibility of milk to the development of oxidized flavor both spontaneously and under induction by 0.1 ppm copper. Under normal conditions one gram per cow per day is sufficient. Analysis of the natural  $\alpha$ -tocopherol content of various forages indicates that the growth stage and preservation methods are important factors governing the amount of natural  $\alpha$ -tocopherol available to the cow.

Research at EU showed that heating milk fat in the presence of nonfat milk solids definitely enhanced its flavor and flavor stability. Heated milk fat, liquid and solid fractions made from it, and vegetable fat were used to make caramels. After six months the flavor of those made with the liquid fraction of heated milk fat was best. A liquid fraction from heated milk fat improved the flavor of commercially manufactured butter cream candy. The solid fraction increased consistency. Heated milk fat also enhanced the flavor of ice milk.

Determination of the peroxide values of the off-flavored foam-spray dried whole milk powders produced during the summer months failed to show that oxidative changes during drying were responsible for flavor deterioration. This correlated well with a later finding suggesting that the summer flavor developed during drying even though the air in the vicinity of the dryer was very low in ozone. The equipment has now been installed to permit the direct collection of volatiles in the atmosphere entering the Dairy Products Laboratory spray dryer. The micro-particles will be collected and analysis made by nuclear activation techniques.

### C. Color, Texture and Other Quality Factors

Progress is being made in control of sedimentation which occurs in high-temperature short-time (HTST) sterilized milk products. Sterilizing before concentration reduces sedimentation of denatured proteins. The addition of hexametaphosphate and orthophosphate to milks before sterilization also reduces the amount of sedimentation in milks sterilized both before and



after concentration.

Quantitative determination of tryptophan in milk proteins were continued to study the relation of tryptophan content to antigenic properties. Two basically different methods of analyzing for tryptophan gave good agreement on 31 proteins including the A, B and C variants of  $\beta$ -lactoglobulin,  $\alpha_s$ - and  $\beta$ -casein, the A and B variants of  $\kappa$ -casein and  $\alpha$ -lactalbumin. No difference was found in tryptophan content of any of the variants.

$\kappa$ -Casein was found to have one tryptophan residue per mole in contrast to two reported in the literature.

Pepsin digests of  $\beta$ -lactoglobulin were used to prepare rabbit antisera to test for possible development of new antigenic specificity. Antibodies for  $\beta$ -lactoglobulin were found in hydrolyzates up to and including the 15-hour digests.

From a group of 24 individuals who gave positive allergic reactions to skim milk by direct skin tests, 7 individuals provided sera which induced high sensitivity to skim milk in passive transfer sites in the allergic groups. Sensitivity was directed most strongly to  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin.

In a study to develop improved assay methods rabbits were sensitized to crystalline ovalbumin via the footpads and subsequently challenged, severe shock occurred 10-14 days after the sensitization. However, assay for passive cutaneous anaphylactic antibody in the sera did not correlate well with the systemic reactions. The poor correlation between the titer of the antiserum and the appearance of systemic symptoms suggests that different antibody systems may be involved in these two phenomena.

#### D. Microbiology and Toxicology

1. Microbial spores. Preliminary experiments with a microwave generator suggests that frequencies near 22,000 mc per second may be lethal for microbial spores. However, reproducibility of these results has not been satisfactory partly because of assay method limitations and partly because of the low maximum power output which is available. The lethal effect is envisioned as occurring through denaturation of vital specific spore structures which would resinate with certain microwave frequencies producing micro-heating within the spores.

Results of research on techniques of controlling spore germination indicate that reduction of disulfide groups in the spore coat is involved in the germination process.

In research at the National Dairy Research Institute, Karnal, Punjab, India, various synthetic liquid media were tested for obtaining rapid growth and sporulation of a strain of Bacillus subtilis. A medium in which 93% (by staining) of the cells sporulated was developed and adopted for further studies. Maximum sporulation was obtained 33 hours after transfer into the

final culture flask and the total maximum growth obtained was  $2.0 \times 10^8$  cells/ml. Presence of sodium citrate was found to be essential for sporulation and this requirement could not be replaced by malate or sodium acetate. It is planned to use this medium for the studies of factors which influence the process of sporulation and heat resistance of spores.

2. Bread yeast activity. Contract research at the University of Wisconsin on the effects of nonfat dry milk on bread yeast has been completed. This work has shown that continuously produced yeast ferments which incorporate nonfat dry milk are as good as batch ferments for use in continuous baking. Yeast activity was increased by the addition of up to 4% of nonfat dry milk to the yeast food. A process employing a 30-liter continuous fermentor saves operational time, permits accurate control of the sugar level and pH during fermentation and yields a uniform and accurate yeast brew. The data on stimulation of yeast activity by nonfat dry milk, providing improvement of bread quality, should lead to increased use of nonfat dry milk by the baking industry and help to overcome problems that had been encountered in the early development of continuous breadmaking processes.

#### E. Technology - Process and Product Development

1. Dried milk. A dried nonfat milk with good dispersibility and sinking properties was prepared by the incorporation of either carbon dioxide or nitrogen into the dryer feed at the rate of 0.05 to 0.07 cubic feet of gas per 10 pounds of concentrate. Addition of 0.05 cubic feet of either gas into a dryer feed containing only the liquid fraction of milk fat in such quantities as to give a 1% fat containing beverage on reconstitution yielded a fat-containing milk powder with good dispersibility and sinkability. Previous work has shown that expert tasters have difficulty perceiving significant flavor differences between reconstituted milk containing from 1 to 4% butterfat especially when the nonfat solids of the low-fat milk is raised to compensate for the lower fat value.

Progress was made in the study of the compounds responsible for the fat-derived stale flavor of whole milk powder. Butteroil stored in an inert atmosphere was subjected to a low pressure steam distillation; at 50°C. and 1 mm. pressure the off-flavors could be removed completely from stale butteroil. Gas-liquid chromatography indicated at least 40 volatile compounds in the distillate. Eleven compounds accounted for the bulk of the volatile material. The taste panel applied "stale" and "unclean" criticisms to the more volatile fractions.

The methods developed by Dr. D. P. Schwartz, of the Dairy Products Laboratory (DPL) have been used in PL-480 sponsored research at Technical University, Berlin, West Germany, to study the carbonyls in fresh butterfat and samples of spray-dried milk powder stored under various conditions. Significant change in the carbonyl fraction during the storage of whole milk powder only occurs if the product is exposed to air during storage. Under these conditions a marked increase in ketone content is noted. These findings tend to indicate that the development of "stale" flavor in DPL-developed whole

milk powder during storage is probably not associated with marked changes in its carbonyl content.

In research at EU, a recording microbalance has permitted precise determination of the adsorption-desorption isotherms for water vapor on spray-dried and foam-spray-dried whole milk, skim milk and whey powders. Sample is 10 mg. The results demonstrate that conventionally spray-dried powders are not more hygroscopic than comparable foam-spray-dried materials. Powders held in water vapor containing atmospheres and then redried undergo distinct structural changes in the powder particles, affecting the porosity of the powders. These findings were made by gas diffusion techniques. The immersion of dried milk powders in ammonium sulfate solutions results in the leaching out of lactose and salts of milk while the particles retain their structure. More than 80% of the total calcium may be removed from foam-spray-dried skim milk powders. The skeletal structure which remains after removal of calcium lactose from milk powders constitutes evidence for some sort of protein-protein interaction which occurs during the dehydration step.

The best available mathematical model describing the continuous vacuum foam drying process was used to predict conditions necessary for year-round operation of the vacuum foam dryer at economical rates. On the milks available (winter and spring transitional) there was good agreement between the predicted and observed results. At the end of their research phase under this project the results, some of which were reported earlier, are as follows:

There has been produced in the pilot plant by a continuous process a product which reconstitutes with (a) good flavor, texture and appearance, (b) excellent cold water (40°F.) dispersibility which remains unaltered on storage, (c) no oxidative damage, and (d) retention of good beverage quality for a year under ordinary refrigeration.

The process has been operated experimentally year-round in the pilot plant at economic rates despite seasonal variations in milk foaming properties. Plans are now being made for a retail market test which is a necessary step in the encouragement of commercial development of this unique process.

2. Cheese. The laboratory study of the preparation of low-fat cheese showed that the flavor and texture of the experimental cheeses were markedly affected by the amount of milk fat, moisture content, intrinsic milk lipases and rate and extent of acid development. The manufacturing procedures most directly related to the control of moisture, acidity and flavor were the homogenization of the milk fat, the fortification of cheese milk with dry skim milk solids, the type and amount of starter, the curd size and rate and extent of curd cooling and the temperature and type of cheese pressing. Pilot plant scale operations have yielded some cheeses of satisfactory quality but reproducibility has not been sufficiently good. Modification of procedures and slight changes in composition promise better control of variations. Shelf life beyond 8 to 10 weeks has been accomplished by blending and processing the



natural cheese. Tests by the Market Potential Branch (PRS) indicate that the new cheese should have good consumer acceptance. Since this low-fat cheese (5 - 7% milk fat and 55 - 59% moisture) has fewer calories than standard cheeses, it may be attractive to calorie counters.

In a study to determine whether *Salmonella* can survive during the manufacture of cottage cheese, selected strains of *Salmonella* were added to the cottage cheese milk. The analysis of 16 lots of cheeses made with 6 *Salmonella* test strains showed that a cooking temperature of 125°F. or above was required to destroy *Salmonella*. The rate and extent of acid produced by the lactic acid forming starter bacteria materially affected the destruction of *Salmonella*. Strains of *Salmonella* added to cottage cheese after manufacture continued to survive in the cheese without appreciable reduction in numbers for a 2- to 3-week period, which is a normal shelf life. These results provide reasonable assurance that *Salmonella* will not survive in cottage cheese if the cooking temperature is 125°F. or higher. However, if the cooking temperature is less than 125°F., the milk or milk fortified with nonfat dry milk should be pasteurized.

Research under a PL-480 grant at Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, showed that an acceptable cheese can be made from buffalo milk to which nonfat dry (cow's) milk has been added to reduce the milk fat content.

In the research at National Dairy Research Institute, Punjab, India, milk clotting enzyme from selected strains of *Bacillus subtilis* were found to be sufficiently heat and pH stable for use in cheese-making. They also have proteolytic and lipolytic activities. They were used as rennet substitutes in making Cheddar cheese. The quality of the cheese and the chemical changes in it were practically the same as those in cheese made with rennet. Enzymes from *B. subtilis* are preferred over those from *B. megatherium* or *B. cereus*.

3. Removal of radionuclides from milk. Research under a U. S. Public Health Service contract with the Producers Creamery Company, Springfield, Missouri, on three full-scale runs showed that a combined anion-cation fixed resin bed system successfully removed about 99% of iodine-131 and 95% of strontium-85 from whole milk previously spiked with these isotopes. Flavor scores of the treated milk averaged 35.9 with the ADSA scoring system. The major flavor defect was described as "chemical" or "medicinal." Manufacturing costs for milk processed in this way was 6.3 cents per quart of milk exclusive of rent and administrative expense. The employment of a continuous contactor with a moving bed of ion-exchange resin to extract both radioactive iodine and radioactive strontium has been conducted under a contract between the U. S. Public Health Service and the Chemical Separations Corporation, Oakridge, Tenn. to which USDA contributed financial support. The contractor tested this equipment only with water and salt solutions. Although some refinement remains to be worked out with this system, it appears that the continuous contactor system for removal of radionuclides from milk is potentially useful. It is probable that more attention to operating details will be required than is necessary with the fixed-bed system. The fixed-bed system which has been

located at the Producers Creamery Company, has now been dismantled and shipped to the Public Health Service's Laboratory at Montgomery, Alabama. Similarly the continuous bed system will be removed to the Montgomery, Alabama, Laboratories for testing with milk.

Contract research at the Pet Milk Company, Greenville, Illinois, indicates that conventional pasteurization at temperatures from 169 to 172°F. for 16 seconds is inadequate to prolong milk stability to the 8 weeks required for iodine-131 decay. However, storage at temperatures as low as 32°F. materially improved storage stability. Processing temperatures up to 200 to 220°F. for 0.5 to 16 seconds extends milk stability at storage temperature of 40°F. or lower. High temperature processing, however, gave more heat-induced flavor component. Milk pasteurized at 220°F. for 16 seconds and stored at 40°F. or below maintained good quality for 13 weeks. This information should be useful to the market milk industry regardless of any iodine-131 problem.

4. Processing equipment. In grant research at the North Carolina State University, Raleigh, temperature sensors failed within a few seconds when positioned 1.56 inches downstream from the point of steam injection. Acoustic spectra were obtained for 3 different steam injectors operated at 3 processing temperatures in order to determine processing parameters. The acoustic spectra obtained were affected by processing temperature, distance of the sensor from the steam injection nozzle, injector design and liquid pressure downstream from the injector.

In 3 trials in commercial establishments in which foam-spray drying with liquified carbon dioxide was employed on whey, difficulties were encountered in removing the powder from the dryer and in no case was the powder produced as good as that produced in the Dairy Products Laboratory. The problem appears to be due to aggregation as the partially-dried droplets hit the Dairy Products Laboratory dryer wall, adhere, and are swept back into the drying air by the revolving sweep. Commercial driers are designed to prevent sticking to any surface. The data suggests that the desirable qualities of foam-spray dried products in the Dairy Products Laboratory pilot plant may be due to a combination of puffing and in-dryer agglomeration.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

- Basch, Jay J., and Timasheff, S. N. 1967. Hydrogen ion equilibria of the genetic variants of bovine  $\beta$ -lactoglobulin. Arch. Biochem. Biophys., 118, pp. 37-47.
- Berlin, E., Kliman, P. G., and Pallansch, M. J. 1967. Surface features of foam-dried milk powder granules from krypton adsorption measurements. J. Dairy Sci., 50, pp. 659-663.
- Berlin, E., Lakshmanan, S., Kliman, P. G., and Pallansch, M. J. 1967. Isolation and physicochemical characterization of a lipoprotein fraction from bovine milk. Biochemistry, 6, pp. 1388-1394.
- Bernardi, A., and Bernardi, G. 1966. Studies on acid hydrolases. III. Isolation and properties of spleen acid ribonuclease. Biochim. Biophys. Acta, 129, pp. 23-31.
- Bernardi, G., Bernardi, A., and Chersi, A. 1966. Studies on acid hydrolases. I. A procedure for the preparation of acid deoxyribonuclease and other acid hydrolases. Biochim. Biophys. Acta, 129, pp. 1-11.
- Bernardi, G., and Cordonnier, C. 1965. Mechanism of degradation of DNA by endonuclease I from escherichia coli. J. Mol. Biol., 11, pp. 141-143.
- Boroff, D., Fleck, V., Townend, R., and Das Gupta, B. R. 1966. Ultra-centrifugal analysis of the crystallin toxin and isolated fractions of clostridium botulinum type A. J. Biol. Chem., 241, p. 5165.
- Cerbulis, J., and Ard, J. S. 1967. Method for isolation and detection of dioctyl phthalate from milk lipids. J. Assoc. Offic. Anal. Chemists, 50, pp. 646-650.
- Chersi, A., Bernardi, A., and Bernardi, G. 1966. Studies on acid hydrolases. II. Isolation and properties of spleen acid phosphomonoesterase. Biochim. Biophys. Acta, 129, pp. 12-22.
- Delfour, A., Alais, C., and Jolles, P. 1966. Structure of cow's  $\kappa$ -caseinoglycopeptide: the N-terminal octadecapeptide. Chimia(Aarau), 20, pp. 148-150.
- Gorbunoff, M. J. 1967. Exposure of tyrosine residues in protein: reaction of cyanuric fluoride with ribonuclease,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. Biochemistry, 6, pp. 1606-1615.

- Hemken, R. W., and Vandersall, J. H. 1966. Feasibility of an all-silage forage program. *J. Dairy Sci.*, 50, pp. 417-422.
- Herskovitz, T. T. 1966. On the conformation of caseins. Optical rotatory properties. *Biochemistry*, 5, pp. 1018-1026.
- Hoagland, P. D. 1966. Acylated  $\beta$ -caseins: electrostatic interactions and aggregation. *J. Dairy Sci.* 49, pp. 783-787.
- Kalan, E. B., and Basch, J. J. 1966. Preparation of goat  $\beta$ -lactoglobulin. *J. Dairy Sci.*, 49, p. 406.
- Kalan, E. B., Greenberg, R., and Thompson, M. P. 1966. Analysis of proteolytic digests of genetic variants of  $\alpha_{s1}$ -casein. *Arch. Biochem. Biophys.*, 115, pp. 468-477.
- Kumosinski, T. F., and Timasheff, S. N. 1966. Molecular interactions in  $\beta$ -lactoglobulin. X. The stoichiometry of the  $\beta$ -lactoglobulin mixed tetramerization, *J. Am. Chem. Soc.*, 88, p. 5635.
- Leach, B. E., Blalock, C. R., and Pallansch, M. J. 1967. Kinin-like activity in bovine milk. *J. Dairy Sci.*, 50, pp. 763-764.
- Parkash, S., and Jenness, R. 1967. Status of zinc in cow's milk. *J. Dairy Sci.*, 50, pp. 127-134.
- Parks, O. W., Schwartz, D. P., Nelson, K., and Allen, C. 1967. Evidence for kynurenine in milk. *J. Dairy Sci.*, 50, pp. 10-11.
- Peterson, R. F., Nauman, L. W., and Hamilton, D. F. 1966. Amino acid composition of six distinct types of  $\beta$ -casein. *J. Dairy Sci.*, 49, pp. 601-607.
- Phillips, N. I., Jenness, R., and Kalan, E. B. 1967. Reactivity of sulfhydryls in several  $\beta$ -lactoglobulins. *Arch. Biochem. Biophys.*, 120, pp. 192-197.
- Pikkarainen, J., and Kulonen, E. 1965. The fractionation of various gelatin preparations with ion-exchange resins. *Suomen Kemistilehti B* 38, pp. 226-230.
- Schwartz, D. P., Brewington, C. R., and Burgwald, L. H. 1967. Rapid quantitative procedure for removing cholesterol from butterfat. *J. Lipid Res.* 8, p. 54.
- Schwartz, D. P., Burgwald, L. H., and Brewington, C. R. 1966. A simple quantitative procedure for obtaining the unsaponifiable matter from butteroil. *J. Am. Oil Chemists Soc.*, 43, pp. 472-473.



- Thompson, M. P. 1966. DEAE-cellulose-urea chromatography of casein in the presence of 2-mercaptoethanol. *J. Dairy Sci.*, 49, pp. 792-795.
- Thompson, M. P., Kalan, E. B., and Greenberg, R. 1967. Properties of caseins modified by treatment with carboxypeptidase A. *J. Dairy Sci.*, 50, pp. 767-769.
- Timasheff, S. N. 1966. Turbidity as a criterion of coagulation. *J. Coll. Interface Sci.*, 21, pp. 489-497.
- Timasheff, S. N., Mescanti, L., Basch, J. J., and Townend, R. 1966. Conformation transitions of bovine  $\beta$ -lactoglobulins A, B, and C. *J. Biol. Chem.*, 241, pp. 2496-2501.
- Timasheff, S. N., Townend, R., and Mescanti, L. 1966. The optical rotatory dispersion of the  $\beta$ -lactoglobulins. *J. Biol. Chem.*, 241, pp. 1863-1870.
- Timasheff, S. N., Townend, R., and Perlmann, G. E. 1967. The circular dichroism of phosvitin. *J. Biol. Chem.*, 242, pp. 2290-2292.
- Townend, R., Kumosinski, T. F., and Timasheff, S. N. 1966. The circular dichroism of the  $\beta$ -structure of poly-L-lysine. *Biochem. Biophys. Res. Commun.*, 23, pp. 163-169.
- Weil, L., and Timasheff, S. N. 1966. On the enzymatic activity of trypsin autolysis products. *Arch. Biochem. Biophys.*, 116, pp. 252-254.
- Woychik, J. H., Kalan, E. B., and Noelken, M. E. 1966. Chromatographic isolation and partial characterization of reduced  $\kappa$ -casein components. *Biochemistry*, 5, pp. 2276-2282.
- Zittle, C. A. 1966. Precipitation of casein from acidic solutions by divalent anions. *J. Dairy Sci.*, 49, pp. 361-364.
- Zittle, C. A., and Custer, J. H. 1966. Identification of the  $\kappa$ -casein among the components of whole goat casein. *J. Dairy Sci.*, 49, pp. 788-791.

### Flavor

- Abdel-Rahman, S., and Virtanen, A. I. 1965. On the composition of the blood and plasma of test cows fed on a purified diet with urea and ammonium salts as the sole sources of nitrogen. *Suomen Kemistilehti B* 38, pp. 280-281.
- Dimick, P. S., and Walker, N. J. 1967. Efficiency of laboratory steam deodorization in quantitative recovery of aliphatic delta-lactones from butteroil. *J. Dairy Sci.*, 50, pp. 97-99.



- Edmondson, L. F., Avants, J. K., Douglas, F. W., Jr., and Easterly, D. G. 1966. Phosphatase and lipase reactivation in high-temperature short-time sterilized milk. *J. Dairy Sci.*, 49, p. 708.
- Fox, K. K., Harper, M. K., Holsinger, V. H., and Pallansch, M. J. 1967. Effects of high-heat treatment on stability of calcium caseinate aggregates in milk. *J. Dairy Sci.*, 50, pp. 443-450.
- Homer, D. R., and Virtanen, A. I. 1967. Cow's milk cholesterol - studies on the milk of cows on normal and protein-free feeds. *Milchwissenschaft*, 22, p. 1.
- Kurtz, F. E., 1967. Possible inclusion of artifacts in flavors recovered directly from dried whole milk. *J. Dairy Sci.*, 50, pp. 814-817.
- Parks, O. W., Schwartz, D. P., Keeney, M., and Damico, J. N. 1966. Isolation of  $\Delta^7$ -cholesten-3-one from butterfat. *Nature*, 210, no. 5034, pp. 417-418.
- Parks, O. W., Schwartz, D. P., Nelson, K., and Allen, C. 1967. Evidence for kynuronine in milk. *J. Dairy Sci.*, 50, pp. 10-11.
- Schwartz, D. P., and Brewington, C. R. 1966. Methods for the isolation and characterization of constituents of natural products. I. Derivatives of alcohols with pyruvyl chloride 2,6-dinitrophenylhydrazone. *Microchem. J.*, 11, pp. 430-436.
- Schwartz, D. P., and Brewington, C. R. 1966. Methods for the isolation and characterization of constituents of natural products. II. Separation of a homologous series of esters of pyruvic acid 2,6-dinitrophenylhydrazone by thin layer chromatography. *Microchem. J.*, 12, pp. 1-6.
- Schwartz, D. P., Burgwald, L. H., and Brewington, C. R. 1966. A simple quantitative procedure for obtaining the unsaponifiable matter from butteroil. *J. Am. Oil Chemists Soc.*, 43, pp. 472-473.
- Syv  oja, E. and Virtanen, A. I. 1965. Fractionation of milk proteins, especially of milk produced on protein-free feed with urea and ammonium salt as the sole source of nitrogen. *Enzymologia*, 29, pp. 205-220.
- Tamsma, A., Kurtz, F. E., Rainey, N., and Pallansch, M. J. 1967. Preconditioning of septums to reduce eluate contamination and anomalous peaks during temperature programming. *J. Gas Chromatog.*, 5, pp. 271-272.
- Virtanen, A. I. 1966. Milk production of cows on protein-free feed. *Science*, 153, no. 3744, pp. 1603-1614.

#### Color, Texture, and Other Quality Factors

- Downey, W. K., and Andrews, P. 1965. Gel filtration applied to the study of lipases and other esterases. *Biochem. J.*, 94, pp. 642-650.

- Downey, W. K., and Andrews, P. 1965. Milk lipases and the association of lipases with casein micelles studied by gel-filtration. *Biochem. J.*, 94, p. 33P.
- Gutfreund, H. 1964. The study of transients in enzyme catalyzed reactions. *Ber. Bunsenges, Physik. Chem.*, 68, pp. 756-759.
- Spies, J. R., and Barron, J. K. 1966. The chemistry of allergens. XVIII. An analysis of CB-1A from castor beans. *Ann. Allergy*, 24, pp. 499-502.
- Spies, J. R. 1967. The chemistry of allergens. XIX. On the number of antigens and the homogeneity of the isolated antigens of fraction CB-1A from castor beans. *Ann. Allergy*, 25, pp. 29-34.

#### Microbiology and Toxicology

- Blankenship, L. C., and Pallansch, M. J. 1966. Differential analysis of sulfhydryl and disulfide groups in intact spores. *J. Bacteriol.*, 92, pp. 1615-1617.
- Garver, J. C., Navarini, I., and Swanson, A. M. 1966. Factors influencing the activation of bakers' yeast. *Cereal Science Today*, 11, pp. 410, 412-414, 416, 418.

#### Technology -- Process and Product Development

- Babbar, I. J., Srinivasan, R. A., Chakravorty, S. C., and Dudani, A. T. 1965. Microbial rennet substitutes - A review. *Indian J. Dairy Sci.*, 18, pp. 89-95.
- Berlin, E., DeVilbiss, E. D., and Pallansch, M. J. 1967. Effect of temperature on porosity of dried whole milk powder granules. *J. Dairy Sci.*, 50, pp. 655-658.
- Cornell, D. G., and Pallansch, M. J. 1966. Counting and sizing fat globules electronically. *J. Dairy Sci.*, 49, p. 1371.
- Farrall, A. W., Selman, R. L., and Hanrahan, F. P. 1966. Cost analysis of foam-spray drying of whole milk. *Trans. ASAE (Am. Soc. Agr. Eng.)*, 9, pp. 771-773.
- Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1967. Denaturation of cottage cheese whey proteins by heat. *J. Dairy Sci.*, 50, pp. 828-832.
- Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1967. Effect of the salts of the lyotropic series on the farinograph characteristics of milk-flour dough. *Cereal Science Today*, 12, pp. 200-203.

- Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1966. Use of foam-spray-dried cottage cheese whey in water ices. J. Dairy Sci., 49, p. 1156.
- Hargrove, R. E., McDonough, F. E., and Tittsler, R. P. 1967. Factors affecting characteristics, composition, and quality of skim milk cheese. J. Dairy Sci., 50, pp. 160-166.
- Hargrove, R. E., McDonough, F. E., and Tittsler, R. P. 1966. New type of ripened low-fat cheese. J. Dairy Sci., 49, pp. 796-799.
- Heinemann, B., Baldi, E. J., Marshall, R. O., Sparling, E. M., Walter, H. E., and Fooks, J. H. 1967. Large scale fixed bed ion-exchange system for removing strontium-90 from fluid milk. II. Compositional studies. J. Dairy Sci., 50, pp. 426-430.
- Holden, T. F., Aceto, N. C., DellaMonica, E. S., and Calhoun, M. J. 1966. Seasonal variation of phospholipids and their influence on the foaming characteristics of concentrated whole milk. J. Dairy Sci., 49, pp. 346-350.
- Huhtanen, C. N., 1966. Effect of ultrasound on disaggregation of milk bacteria. J. Dairy Sci., 49, pp. 1008-1010.
- Isaacks, R. E., Hazzard, D. G., Barth, J., Fooks, J. H., and Edmondson, L. F. 1967. Nutritional evaluation of milk processed for removal of cationic radionuclides. Chemical analyses. J. Agr. Food Chem., 15, pp. 295-299.
- Isaacks, R. E., Hazzard, D. G., Barth, J., Walker, J. P., Fooks, J. H., and Edmondson, L. F. 1967. Nutritional evaluation of milk processed for removal of cationic radionuclides. Feeding studies. J. Agr. Food Chem., 15, pp. 300-304.
- Leach, B. E., Rainey, N. H., Pallansch, M. J. 1966. Physical and chemical analyses of film formed at air-water interface on reconstitution of whole milk powders. J. Dairy Sci., 49, p. 1465.
- Sadler, A. M., Walter, H. E., Hanrahan, F. P., and Edmondson, L. F. 1967. Automated pilot plant for removing radiostrontium from milk. J. Dairy Sci., 50, pp. 268-271.
- Sparling, E. M., Baldi, E. J., Marshall, R. O., Heinemann, B., Walter, H. E., and Fooks, J. H. 1967. Large scale fixed bed ion-exchange system for removing strontium-90 from fluid milk. I. Processing results. J. Dairy Sci., 50, pp. 423-425.

## AREA NO. 2. MEAT UTILIZATION - FOOD

Problem. Livestock production is our greatest single source of farm income. For the past several years over 30 percent of cash receipts from farming were derived from livestock sales. Likewise, the major portion of our land is used to grow livestock feed and forage. Hence, any research which succeeds in stimulating an increase in the consumption of meat and livestock products will have a profound effect on agriculture as a whole. For example, it is estimated that a one-percent increase in meat consumption would require an increase in feed equivalent to 80-million bushels of corn.

The processing of meat and meat products also has an important effect on rural industry and rural employment. About half of our meat supply is derived from packing plants in rural areas. Many of these are small (the state of Pennsylvania alone has over 2,000 registered slaughterers) and cannot hope to maintain their own research facilities. They employ local labor, and their products are transported and sold by local truckers and business men. Thus increases in meat consumption and improvements in meat technology will contribute to increased rural prosperity.

Increases in livestock consumption depend primarily on the availability of wholesome and convenient products for purchase by the American consumer. In recent years the supply of such products has been threatened by the increased danger from infections by bacteria of the genus Salmonella. Research is needed which will lead to the development of meat products which, by virtue of the conditions under which they are processed, can be considered absolutely free of Salmonellae. A similar situation exists with respect to the toxins of Staphylococcus aureus, and to a somewhat lesser extent, those of certain anaerobic spore formers. An obviously important part of our research program must be devoted to providing the scientific "know-how" for the production of safer products for public consumption.

Increases in livestock consumption may be achieved through development of new or improved meat products, or through improved meat processing technology which results in lower costs. In addition, increases in the value of hides, animal fats, and renderers' proteins will benefit the livestock industry by providing additional revenues which could permit reduction in meat prices (thus stimulating consumption) or which could flow back through the marketing channels in whole or in part to livestock growers and feed producers. For example, it is estimated that loss of the market for hides would cause an increase of meat prices that would result in a decrease of 2 percent in meat consumption. Such a decrease would eliminate a market for feed equivalent to 160 million bushels of corn. Conversely, an increase in hide values would operate in the opposite direction and would result in greater income to the livestock industry and in increased utilization of feed grains.

Increased livestock consumption required both basic and applied research. Applied research is the forerunner of commercial practice and is an



indispensable element in successful development. But applied research depends on new knowledge which must be developed in fundamental studies. Our supply of fundamental knowledge must be maintained and expanded if applied research is to be effective and fruitful.

For the reasons given above, research which succeeds in increasing net consumption can have a powerful effect on American agriculture. The potential effect may be assessed from the facts that meat has a high elasticity demand (a 1-percent drop in retail prices will result in a 0.7-percent increase in consumption); the production of one pound of livestock requires the equivalent of 7 to 8 pounds of feed grains; and the present United States consumption of meat (174 lb/person in 1964) is still below that of Australia (234 lb.), New Zealand (222 lb.), or Uruguay (234 lb.). Economists predict that the 1967 United States consumption will be slightly lower than in 1964.

Attaining increased meat consumption and providing new technological information for small processors will require a vigorous and balanced research program. There is need for more applied research on processing and preservation, including expanded studies on increasing the efficiency of sausage production and on new dried or semi-dried and ready-to-eat products. Of even greater importance is the need for more basic research on the physical, chemical, and microbiological properties of meat to provide a fund of knowledge for future technological improvements.

#### USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists, microbiologists, and food technologists engaged in both basic studies and in the application of known principles to the solution of problems in the processing of meat and meat products. The Department's research facilities are located at Beltsville, Maryland, and at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 25.5 scientist man-years including 6.3 scientist man-years of contract and grant research. This effort is applied as follows:

(a) Research on chemical composition and physical properties involves 3.2 scientist man-years at Wyndmoor. A research contract at Louisiana State University provided for 1.0 scientist man-year to study the modification of muscle connective tissue constituents and their relationship to tenderness. This was completed during the reporting year.

(b) Flavor research is conducted at Wyndmoor, and involves 4.0 scientist man-years. Additional research under a PL-480 grant is in progress at Gdansk, Poland, on antioxidant components of wood smoke used in meat curing (5 years, 1963-1968).

(c) Research on color, texture and other quality factors involves 4.5 scientist man-years on investigations related to oxidation of tissue lipids at Beltsville, Maryland, and on fundamental studies of muscle pigment

chemistry at Wyndmoor, Pennsylvania. The relationship between heme pigments and oxidative rancidity in cooked and frozen meats is being studied at Florida State University under a grant involving 0.7 scientist man-year.

Another grant, for research on the noncarbonyl compounds associated with rancid meat, involves 0.5 scientist man-year effort at Rutgers University. Additional research is in progress under a PL-480 grant at British Food Manufacturing Industries Research Association, Leatherhead, Surrey, England, on specific reducing systems in pork muscle (5 years, 1964-1969).

(d) Research on microbiology and toxicology of meat and meat products involves 4.5 scientist man-years at Beltsville, Maryland. Contract research at Iowa State University, involving 1.2 scientist man-years, is concerned with a study of the fungi associated with cured meat. In addition, a recently-negotiated grant at the University of California, Davis, provides for a study of the effect of indigenous microflora on growth and toxin production of Staphylococcus aureus and undesirable Clostridia in cured meats. Research is also conducted under a PL-480 grant at Central Institute for Nutrition and Food Research, T.N.O., Utrecht, Netherlands, on the use of protozoa to detect harmful substances in meat (5 years, 1965-1970).

(e) Technology - process and product development involves 3.0 scientist man-years at Wyndmoor, Pennsylvania. A research contract at Michigan State University involving 0.8 scientist man-year is for the purpose of developing new smoked meat products. Another contract provides 0.7 scientist man-year effort for research at the University of Missouri to develop new meat products for freezing.

The development of new ready-to-eat meat products suitable for production in small, rural industries is being investigated under a contract (0.4 scientist man-year) at Southern University, Baton Rouge, Louisiana, and the reactions of muscle proteins as they relate to the thermal effects of meat processing and large-scale institutional cookery is being studied under a contract (0.5 scientist man-year) with Cornell University, Ithaca, New York. Contract research at Pennsylvania State University, University Park, involves 0.5 scientist man-year on development of fabricated meat products, including new lamb and mutton products.

In addition, research sponsored by the Department under PL-480 grants is in progress at Taiwan Provincial Chung Hsing University, Taichung, Taiwan, on preparation of new semi-dehydrated types of fried meat products (3 years, 1964-1967), and University of Helsinki, Helsinki, Finland, on influence of fats on flavor and aroma of fried sausage (5 years, 1963-1968).

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 28.0 scientist man-years is devoted to this area of research.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

### A. Chemical Composition and Physical Properties

Freshly prepared pre-rigor muscle myosin of high purity was prepared from beef, pig and rabbit longissimus dorsi muscles. The enzymatic activity of these myosins towards adenosine triphosphate showed differences which are attributed to species characteristics and appears to affect meat quality. The method for measuring enzymatic activity was improved and the reproducibility in estimates with this new method is  $\pm 2\%$ . In contract research at Louisiana State University, histochemical studies showed that the amount and character of reticular fibers and degree of fiber contractions in muscle was related to tenderness. The content of acid- and salt-soluble collagens and total muscle calcium was not related. Other experiments dealt with the relation of the amount of acid mucopolysaccharides in bovine tissue to tenderness and to the movement of calcium ion in muscle tissue.

### B. Flavor

A water extract of beef was dialyzed and flavor precursors were fractionated on polyacrylamide gel. One fraction had a strong roast beef aroma on pyrolysis and contained all of the threonine, proline, glutamic acid, alanine, valine, leucine and isoleucine in the diffusate. Water extracts of veal, pork and lamb showed a difference among the pyrolysis aromas of the diffusate, but exact identification of species could not be made. Fat played a major role in recognition of lamb flavor but was of less importance in the case of pork and beef. A standard fractionation procedure has also been devised and quantitative analytical methods for sugars, amino acids and other compounds have been established.

In research on flavor and aroma contributing factors in wood smoke, additional components identified were acetol, 2-cyclopentenone, methyl cyclopentenone and, tentatively, veratrol. When ferulic acid (an acid formed by the decomposition of lignin) was thermally decomposed, the phenolic products were the same as those in wood smoke. The fractionation of wood smoke by gas liquid chromatography permits the elimination of components not required for flavor and greater attention can thus be applied to the flavor components. Combination of a few readily available phenols gives a smoke flavoring mixture scarcely distinguishable from smoke condensates.

Of interest in the research under a PL-480 grant at the Technical University, Gdansk, Poland, is the finding that the compounds, 4-methyl-2-propyl-phenol, 3,4-dimethyl-6-ethylphenol, resorcylic acid, and 2,4,6-trimethylphenol are the most active antioxidants in smoke. Also, temperatures no higher than 400°C. in the smoke generator were found to be most effective, both from the standpoint of flavor development and antioxidant activity.

The investigations at Wyndmoor and Gdansk are, accordingly, providing significant and complementary results.



### C. Color, Texture and Other Quality Factors

1. Stability. In studies of stored pork, bacon sides and mixtures of ground tissue fat and lean were treated with several salts before freezer storage. The salts were sodium chloride, sodium nitrite, calcium chloride, sodium nitrate, magnesium chloride, lithium chloride, potassium chloride, sodium sulfate and potassium sulfate. All salts showed some pro-oxidative effect. Sodium chloride-sodium nitrate mixtures have the greatest effect. In the case of sodium chloride the chloride ion did not appear to be responsible for the oxidative effect and sodium nitrite-heme combinations had an oxidative effect which was independent of sodium chloride.

The hydroperoxides of an oxidized lard were effectively reduced or otherwise decreased by stannous, ferrous and cuprous ions. However, these ions formed carbonyls in their action on the fat. These metallic ions have a remarkable oxidizing action on alcohols by producing significant amounts of monocarbonyls.

In grant research at Rutgers University, the volatiles from four samples of beef--laboratory boiled beef, pilot plant boiled beef, freezer-dried and boiled beef, and freeze-dried beef--yielded dissimilar gas chromatograms. Some volatiles appeared to contain sulfur. The volatile flavor components gave more than 100 peaks on an analytical gas chromatogram.

2. Meat pigments. Kinetic studies of the reaction of the heme pigments of meat with nitrite ion and various reductants to produce nitrosylmyoglobin (the undenatured cured meat pigment) indicated that a reductant-nitrite oxide complex was an intermediate in the reaction. The reductants were: ascorbate, cysteine, nicotinamide, adenine dinucleotide and hydroquinone. These studies seek to elucidate the formation of nitrosylmyoglobin and thus far have been carried out in vitro. The reaction mechanisms during actual processing are not the same, possibly because of participation by enzymes in the meat.

In grant research at the Florida State University, studies continued on enzymatic pathways for metmyoglobin reduction in meat. Monosodium glutamate and the glycolytic intermediates, fructose diphosphate or glycerol 3-phosphate, were found effective in promoting the metmyoglobin reduction in ground beef, aiding in good color retention.

Studies under a PL-480 grant at the British Food Manufacturers Research Association, Leatherhead, Surrey, England, of the mechanism of nitrosylmyoglobin formation showed that myoglobin was rapidly oxidized to metmyoglobin. The nitrosyl group was transferred from nitrosylferricytochrome-c to reduced metmyoglobin. The reduction and transfer were induced by nicotinamide adenine dinucleotide-cytochrome-c reductase. The nicotinamide adenine dinucleotide is oxidized in the process. Addition of succinate and nicotinamide to simulated cures accelerated color formation and suggests that a succinate-nicotinamide additive could be used beneficially in curing.



#### D. Microbiology and Toxicology

1. Microbial lipases. Study of the synthesis of Pseudomonas fragi lipase indicates that the enzyme is elaborated by cell envelope preparations. Concomitant with this elaboration, 2.6% of the envelope protein is released, indicating that the release of lipase is a part of the general release of several proteins. The ability of the lipase of Staphylococcus aureus to attack both the one- and two-positions of triglycerides is apparently due to broad specificity rather than to a mixture of two or more specific enzymes. Efforts to separate the enzymatic activity by ultracentrifugation and by column chromatography were unsuccessful. Four groups of microorganisms were distinguished on the basis of their action on monocarbonyls in rancid fat. Group I had little or no effect. Group II decomposes 2,4-dienals. Group III decomposes 2,4-dienals and 2-enals. Group IV decomposes the monocarbonyl fraction completely. The destruction of carbonyls by microbial activity suggests that selected bacteria might be employed to control the development of rancidity which sometimes occurs in fermented sausages.

2. Microbial metabolism. Combinations of sodium chloride, sodium nitrate, and sodium nitrite, in the concentration allowable in cured meat, reduced toxin production by Staphylococcus aureus, but not to a significant degree. Low temperatures, however, (below 10°C.) were very effective in reducing toxin production. Mutant cells, produced in the laboratory by artificial means, which were capable of growth at 5°C., were unable to produce enterotoxin at this temperature even when the amount of growth was comparable to that obtained at 37°C. The toxin is elaborated early in the growth cycle. There is evidence that Staphylococci caused more food poisoning cases attributed to meat than any other microorganisms and are involved in twice as many instances of food poisoning as Salmonellae.

In research under a PL-480 grant at Central Institute for Nutrition and Food Research, Utrecht, Netherlands, protozoa have not appeared to be very sensitive to toxins of microbial origin appearing in food; therefore, attempts were made to use easily grown small animals for detecting bacterial and fungal toxins and histamine phosphate. Water fleas were found sensitive to 0.01% histamine and 0.15 microgram/ml. aflatoxins B and G. The response time was 1 to 45 hours depending on the concentration. This sensitivity is the highest recorded so far. Brine shrimp were less sensitive to these two toxins, responding to 0.1% and 1.5 microgram/ml. respectively in 1-3 days. Guppies were sensitive to botulinus E (10 MLD/ml.), histamine (0.1%) and aflatoxin (0.15 $\mu$ /ml.).

Contract research at Iowa State University suggests that the manufacturer of "country cured" hams, fermented sausages and "country cured" bacon brings about a habitat which leads to similar fungal flora regardless of the geographical location of the processor. Lipolytic and proteolytic changes in fermented sausages examined at various stages of maturity showed that the maximum change in free fatty acids occurred between the fresh and intermediate stages of ripening. Free fatty acid formation appears to be highly important to flavor development. These studies should provide useful

information on the feasibility of adding known microorganisms to meats during curing.

#### E. Technology - Process and Product Development

1. Processing research. In new studies using modern methods of physical analyses, it was shown that the presence of liquid fat, whether added as such or produced through melting, contributes to instability in sausage emulsions. Since it is known that stable meat emulsions are not always formed even though temperatures below 65°F. are maintained, these results indicate that maximum temperature for successful emulsification cannot be arbitrarily fixed but depends on the melting characteristics of the fat used and the emulsifying capacity of the lean raw materials. As determined by differential thermal analysis, tissue fats melt primarily in two ranges, 48°-55° and 68°-88°F. for pork fat, and 45°-57° and 63°-99°F. for beef fat. Tests with low melting fat fractions resulted in emulsion instability at critical levels of liquified fat without relation to temperature per se.

Research during the past year has shown that the maximum temperatures attained during the processing of canned hams can be estimated at a later date by determining the amount of acid phosphatase remaining in the tissue. The accuracy obtained is greater than that possible by existing methods and thus forms a basis for improved regulatory procedures.

Studies of sausages produced with microbial inocula showed that a mixed Lactobacilli-Micrococci starter produced sausages of superior quality in a shorter time than uninoculated controls or sausages inoculated with pure cultures. Of particular interest was the ability of Micrococci to lower peroxide numbers. This research is conducted at the University of Helsinki, Finland, under a PL-480 grant.

2. New products. Contract research at Michigan State University showed that the phenol content correlated best with observed smoked aroma. The results of chemical and organoleptic analyses showed that phenol rather than acid content was significantly correlated with the perception of smoked flavor in bologna. Good results were obtained in getting a controlled uniform smoked flavor in bologna by incorporating smoked nonfat dried milk. Another new product developed was a meat loaf produced from smoked pork trimmings.

In PL-480 sponsored research at Taiwan Provincial Chung Hsing University, Tyndallization (intermittent sterilization) was effective in destroying both Staphylococcus aureus and PA 3679 at low temperature. This points to a commercial possibility for producing sterile, dehydrated meat by a novel application of Tyndallization.

Contract research at the University of Missouri showed that thick slices of "roastbeak," meat pie, beef with noodles, beef with spaghetti, and Swiss steak can be held in frozen storage for 12 months without marked decrease in quality. Storage temperatures were -5° to 0°F. for all products except beef

pie which was held at  $-14^{\circ}$  to  $-18^{\circ}\text{F}$ . A study of gravies indicated that a pectin-calcium chloride thickening agent resisted freezer damage better than several starches tested. The "roaststeak" material is a distinctive product developed by the contractor and has great potential in the restaurant trade.

Contract research at Cornell University on the reactions of muscle protein and thermal effects of meat processing has emphasized use of a calorimeter for precise measurements involving heat of transitions in meat fat. A micro-calorimeter was modified by the addition of an effluent analyzer which will be used in characterizing gaseous products formed in cooking.

Studies conducted under contract at Southern University dealing with production of barbecued-like products in small rural industries, indicated that considerable economies of time were possible when microwave heaters were used in comparison with conventional methods. An interesting point for further investigation was the finding that microwave heating resulted in greater fat losses than conventional heating methods.

## PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

### Chemical Composition and Physical Properties

- Gothard, R. H., Mullins, A. M., Boulware, R. F., and Hansard, S. L. 1966. Histological studies of post-mortem changes in sarcomere length as related to bovine muscle tenderness. J. Food Sci., 31, pp. 825-828.
- Mozersky, S. M., Pettinati, J. D., and Kolman, S. D. 1966. An improved method for the determination of orthophosphate suitable for assay for adenosine triphosphatase activity. Anal. Chem., 38, pp. 1182-1187.
- Swift, C. E., Sulzbacher, W. L., Mozersky, S. M., Pettinati, J. D., and Kolman, S. D. 1966. Protein changes in producing cured pork. Tehnol. Mesa, 7, pp. 194-199.

### Flavor

- Wasserman, A. E. 1966. The composition of meat flavor. Natl. Provisioner, 155, no. 23, pp. 23-25, 40.

### Color, Texture, and Other Quality Factors

- Fox, J. B. Jr., Townsend, W. E., Ackerman, S. A., and Swift, C. E. 1967. Cured color development during frankfurter processing. Food Technol. 21, pp. 386-392.
- Gaddis, A. M., Ellis, R., Currie, G. T., and Thornton, F. E. 1966. Carbonyls in oxidizing fat. X. Quantitative differences in individual aldehydes isolated from autoxidated lard by mild methods of extraction. J. Am. Oil Chemists Soc., 43, pp. 242-244.
- Herz, K. O., and Chang, S. S. 1966. An apparatus for the isolation of volatile compounds from foods. J. Food Sci., 31, pp. 937-940.
- Watts, B. M., Kendrick, J., Zipser, M., Hutchins, B., and Saleh, B. 1966. Enzymatic reducing pathways in meat. J. Food Sci., 31, pp. 855-862.
- Zipser, M. W., and Watts, B. M. 1967. Ascorbate and tripolyphosphate in cured, cooked, frozen pork. J. Agr. Food Chem., 15, pp. 80-82.

### Microbiology and Toxicology

- Doerr, R. C., Wasserman, A. E., and Fiddler, W. 1966. Composition of hickory sawdust smoke. Low-boiling constituents. J. Agr. Food Chem., 14, pp. 662-665.



Fiddler, W., and Doerr, R. C. 1966. Low temperature discontinuity of retention times with carbowax 20M. J. Chromatog. 21, pp. 481-482.

Fiddler, W., Doerr, R. C., Wasserman, A. E., and Salay, J. 1966. Composition of hickory sawdust smoke. Furans and phenols. J. Agr. Food Chem., 14, pp. 659-662.

Mencher, J. R. 1966. Purification and characterization of the lipase of Pseudomonas fragi. Ph.D. thesis, Georgetown University.

Smith, J. L., and Alford, J. A. 1966. Inhibition of microbial lipases by fatty acids. Appl. Microbiol., 14, pp. 699-705.

Wasserman, A. E. 1966. Organoleptic evaluation of three phenols present in wood smoke. J. Food Sci., 31, pp. 1005-1010.

#### Technology -- Process and Product Development

Cohen, E. H. 1966. Protein changes related to the temperatures to which hams have been heat-processed. I. Effect of time-temperature on amount and composition of soluble proteins. J. Food Sci., 31, pp. 746-750.

Rongey, E. H., and Bratzler, L. J. 1966. The effect of various binders and meats on the palatability and processing characteristics of bologna. Food Technol., 20, pp. 134-137.

### AREA NO. 3. ANIMAL FATS AND OILS - INDUSTRIAL PRODUCTS

Problem. The 4-1/2 billion-pound-per-year output of inedible fats is one of the major products of the livestock industry. It also is one of major concern, because while production of animal fats has more than doubled in the last 15 years, its principal outlet (in soap) has declined sharply, and is still declining.

The best answer to the question of what to do with huge amounts of fats is to find new uses through utilization research. Already utilization research has played a leading role in finding new uses for over 1 billion pounds of animal fats, and thus helped retain markets for fats. Use of fat in animal feed which was developed through research, has now become the number one domestic use of inedible fats. There is need, however, for new uses not merely to retain or defend markets, but to expand them, and to upgrade the value of animal fats. The organic chemical industry presents a good opportunity for expanded markets, producing as it does a multitude of products--polymers, plasticizers, insecticides, herbicides, lubricants, paper chemicals--totaling 10 billion pounds. Animal fats possess "built-in" properties which make them potentially useful as raw materials to the chemical industry, but research must be done to realize this potential.

An increase of 1 cent per pound in the value of inedible animal fats would provide an additional revenue of \$40 million of the livestock industry. This additional revenue would help the industry and growers in the same way as revenue from other animal products and by-products.

The attainment of an increase in the monetary returns from livestock requires both applied and basic research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research is based on the foundation of fundamental knowledge that is acquired through basic research, and represents the exploitation of this fundamental knowledge. The supply of fundamental facts about composition of animal fats, methods of separation of constituents, preparation of chemical derivatives of constituents and determination of their physical and chemical properties must be maintained and expanded if applied research is to be most effective and fruitful. The need for basic research has been pointed out by the Commission on Increased Industrial Use of Agricultural Products, the National Agricultural Research Advisory Committee and by other responsible groups.

#### USDA AND COOPERATIVE PROGRAM

The Department has a broad program of basic and applied research at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out involving chemistry and physics, aimed at developing new and improved products from fats for use in industry. The total Federal scientific effort devoted to this program is 39.1 scientist man-years, of

which 4.8 are contract and grant research.

The research devoted to studies on chemical composition, physical properties and structure of animal fat amounts to 10.7 scientist man-years, of which 9.0 is at Wyndmoor. This research includes studies of composition of animal fats, the separation of constituents, the preparation of derivatives, the determination of physical and chemical properties of pure compounds and derivatives and, where applicable, computer programming of mathematical methods to expedite evaluation and interpretation of experimental data. Research at Villanova University, Villanova, Pennsylvania, is continuing under a contract to study special interrelationships within triglyceride molecules and a contract on the X-ray investigation of triglycerides, each involving 0.5 scientist man-year. A research grant involving 0.7 scientist man-year at Storrs, Connecticut, provides for the synthesis of pure glycerides.

Research sponsored by the Department under a PL-480 grant (5-years, 1966-1971) is in progress at Technical University, Gdansk, Poland, to study the thermally stable stationary phases for gas-liquid chromatography.

Research on chemical and physical investigations to improve products involves 25.3 scientist man-years at Wyndmoor and 3.1 in contracts and grants, a total of 28.4 scientist man-years.

Studies related to polymers and plastics include the synthesis of experimental monomers or comonomers and the preparation and evaluation of polymeric products derived from animal fat.

In other investigations, compounds derived from animal fats are used as starting material for the preparation of lubricants and lubricant additives.

Research on development of improved synthetic detergents based on animal fats includes preparation, testing of detergent power, and measurement of biodegradability of  $\alpha$ -sulfo fatty acids and their esters, tallow alcohol sulfates and other fat derived materials. The high pressure hydrolysis of animal fats to alcohols without simultaneous chain saturation is being investigated at Swift and Company, Chicago, Illinois, under a research contract involving 1.1 scientist man-years.

At Lehigh University, Bethlehem, Pennsylvania, the interfacial absorption characteristics of wetting agents and detergents are being studied under a research contract involving 0.4 scientist man-year. A contract with Archer-Daniels-Midland, Minneapolis, Minn., equivalent to 1.1 scientist man-years, provides for research on additive chlorination and hydrogenolysis of animal fats.

Exploratory investigations, employing novel and/or improved reaction techniques are conducted at Wyndmoor to provide new chemical derivatives from animal fats. A research grant with the Hormel Institute of the University of Minnesota at Austin, Minnesota, involving 0.5 scientist man-year provides for the investigation of the ozonization of animal fats.

In addition, research sponsored by the Department under PL-480 grants is in progress at the following foreign institutions:

1. Technical University, Gdansk, Poland, on kinetics and thermodynamics of fat autooxidation (5 years, 1964-1969).
2. "L. Torres Quevedo" Scientific Instruments Institute of the "Juan de la Cierva" Foundation for Applied Research, Madrid, Spain, on cocoa butter substitutes from animal fats (5 years, 1962-1967).
3. University of Bombay, Bombay, India, on the preparation and properties of long chain sulfated monoglycerides (5 years, 1964-1969).
4. Universite d'Aix-Marseille, Marseille, France, on hydroxylated fatty derivatives (5 years, 1962-1967).
5. Institut des Corps Gras, Paris, France, on autooxidation of fat at low temperatures (3 years, 1965-1968).
6. Centre National de la Recherche Scientifique, Paris, France, on polyhalogenated fatty acids and their derivatives (2-1/2 years, 1966-1968).

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 2.9 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition, Physical Properties and Structure

In contract research at Villanova University the mathematical study aimed at theoretically determining the overall shape and spacial interrelations within a triglyceride molecule in the liquid state has progressed to the point where computer programs have been written for the nonbonded interaction energy of various conformers of organic molecules related to triglycerides. Since the population distribution of the various conformers of a flexible organic molecule depend on the energies of these conformers, which in turn depend on the nonbonded interaction energies, the method should lead to better understanding of triglyceride molecules. The method chosen for computing the nonbonded interaction energies is a modification of the Hartree self-consistent field procedure.



Under another contract at Villanova, X-ray structure analysis of the  $\beta$ -form of  $\beta$ -11-bromoundecanoyl- $\alpha,\alpha'$ -dicaprin has verified the expected parallel alignment of adjacent chains and the planar configuration of the molecule in the molecular crystals. The unit cell dimensions have been determined and the space group found to be  $P\bar{1}$ . The unit cell is triclinic. This work represents the first time that the structure of a mixed triglyceride has been determined in such detail.

In research at EU the utility of the procedure for the rapid conversion of glycerides to fatty acid methyl esters has been extended to include oils which contain hydroxy or epoxy groups. A similar procedure, but somewhat slower (20 minutes), has been developed for the trans-esterification of cholesteryl esters to methyl esters of cholesterol. This is accomplished without degradation of the steroid moiety, which has been a problem with most analytical methods. An investigation of the partition of lipids between two immiscible solvents from a thermodynamic point of view has been initiated and ternary diagrams for methyl oleate-hexane-acetonitrile and methyl palmitate-hexane-acetonitrile systems have been constructed. The analytical procedures were applied to samples from a number of different sources including human aortas with varying degrees of atheromatosis, to teeth lipids, blood plasma, skin tissue and to various phospholipids, all with satisfactory results.

Under grant research at the University of Connecticut, Storrs, triglycerides of the highest purity have been prepared and furnished to EU. The compositions are POO, OPP and POS, where P is palmitic acid, O is oleic acid and S is stearic acid. Also one diglyceride (1,3 S-P) and one monoglyceride (1 monooleate) were furnished. In somewhat smaller quantities there were prepared in high purity the materials S-S, O-O and LPP, where L is linoleic acid. The techniques and procedures for preparing these high purity mixed acid triglycerides in quantity is expected to be valuable to firms manufacturing standards for gas and thin-layer chromatography.

A computer program developed at EU permitted the testing by computation of several modes of operation of a countercurrent distribution apparatus and the influence of these modes on the effectiveness of separation of two compounds. The program was subsequently revised to make it possible to follow elutions and frontal analyses. The computer was also employed for a variety of other tasks including the correlation of experimental data with theoretical formulae for dielectric properties, for computing amino acid analyses from an automatic amino acid analyzer, and for subdividing an infrared band into two component peaks. Also, a general purpose program has been written to replace many routine desk calculator procedures. Many desk calculations can be saved with this new program.

Research conducted at EU showed that while the proton magnetic resonance (PMR) spectra of  $C_{18}$  acetylenic acids and methyl esters with the carbon-carbon triple bond located at various locations are unexpectedly complex, the PMR spectra can be used to locate the carbon-carbon triple bond in the  $C_{18}$  carbon chain. The spectra of the 5-, 6-, 7-, 8-, 9-, 12-, 13-, and 15-octadecynoic acids are unique. The 10- and 11-octadecynoic acids gave spectra

indistinguishable from each other but different from the spectra of other  $C_{18}$  acetylenic acids. When formic acid, methyl formate and some of their deuterated forms were subjected to infrared analysis it became possible to calculate 90 vibrational frequencies of the eight molecules concerned, with an average error of less than 0.5%. A determination of bond stretching and bond bending force constants permits the calculation of characteristic group frequencies with a high degree of accuracy.

In research at Technical University, Gdansk, Poland, a new gas chromatograph was designed to make precise measurements for determining liquid phase thermostability. An equation, based on the dependence of the vapor pressure on temperature as defined by the Clausius-Clapeyron formula, was derived for describing the dependence of the thermostability on temperature.

## B. Chemical and Physical Investigations to Improve Products

1. Plastics investigations. A number of urethane foams based on polyols from glycerides such as hydroxylated glycerol trioleate, monooleate, lard, neatsfoot and soybean oil were evaluated. The properties of the foam (density, compressive strength and open cell content) were somewhat inferior to foams prepared previously in this laboratory or commercially. 2-Ethylhexyl diepoxy-stearate compares favorably with commercial epoxy-containing plasticizers and could be useful either as a primary plasticizer or in combination with other plasticizers as a plasticizer-stabilizer.

Experimental work on chain transfer in the copolymerization of allyl monomers as vinyl monomers permitted the derivation of a simple general empirical relationship for the estimation of molecular weights of copolymers of a large number of vinyl monomers with many allylic compounds. The result suggests that commercial polymers such as polyvinyl chloride may be modified with mono-functional fatty allylic comonomers to provide polymers whose molecular weights and viscosities would be satisfactory for use in paint films.

2. Detergents investigations. The more soluble fat-based detergents (unsaturated tallow alcohol sulfates and esters of  $\alpha$ -sulfonated acids) solubilized the less soluble tallow-derived detergents (disodium salts of  $\alpha$ -sulfonated acids and saturated alcohol sulfates). This improvement of solubility improves utility at lower washing temperatures and makes them more useful for liquid formulations. Unsaturated ether alcohols prepared from oleyl alcohol and ethylene oxide, propylene oxide or butylene oxide reacted readily with conventional sulfating agents and left most of the double bond untouched. Unsaturated ether alcohol sulfates are very soluble detergents with good lime soap dispersing properties. They may be useful for combining with less soluble tallow based detergents. Past research at EU contributed to the pilot-plant development of phenylstearic acid and hydroxy-phenylstearic acid announced recently by Armour & Co., and Archer-Daniels-Midland Company.

Improved analytical techniques indicate that linear alkylbenzenesulfonate degrades about 75% after 12 days. Sodium isopropyl- $\alpha$ -sulfo-stearate gives 45% of a sulphur-containing residue which is not degraded after 12 days.

Methylene blue analysis, however, shows complete degradation of both compounds in about 5 days. The new methods based on carbon analysis of detergents and degradation products and on analysis for sulfate ion permit better understanding of the products of degradation in streams. These methods should show differences in products of degradation not now evident by present methods of determining pollution by detergents (foam and methylene blue tests).

In contract research at Lehigh University investigation of selected esters of the wetting agent and detergent types was continued. Two wetting agents and two detergents were investigated, sodium hexyl and heptyl  $\alpha$ -sulfopelargonate and sodium methyl  $\alpha$ -sulfomyristate and  $\alpha$ -sulfopalmitate. The rate of adsorption of these has been followed by breaking a vial of activated carbon into a solution of each material. The concentration was followed by measuring the electrical resistance of the solution. The wetting agents reached a constant value of resistance much more rapidly than the detergents. The rate of adsorption on the carbon measured in this way may prove to be a valuable method for making fundamental distinctions between detergents and wetting agents.

In contract research at Swift & Company, Chicago, progress has been made in retaining unsaturation in the hydrogenolysis of animal fats to alcohols; methyloleate was converted to oleyl alcohol with 98% reaction and a hydrogenolysis/hydrogenation ratio of 6. The product of the reaction is potentially valuable as a detergent intermediate. The oleyl alcohol produced may also be useful in cosmetics, textiles and printing inks, and as a general chemical intermediate. The reaction conditions were one hour at 350°C. and 3,000 p.s.i. with a Cr-Zn-Cd-Al catalyst.

Contract research has begun with the Archer-Daniels-Midland Company on the additive chlorination and hydrogenolysis of animal fat. Additive chlorination takes place readily with oleic acid, methyl oleate or oleyl alcohol in methylene chloride at -15° to -20°. Additive chlorination of oleic acid in methanol gave the chloro-methoxystearate. Additive chlorination gives product suitable for hydrogenolysis experiments. The adjacent chlorine atoms in the 9-10 position are fairly unreactive chemically.

In related PL-480 sponsored research at Centre National de la Recherche Scientifique, Paris, chlorination of saturated fatty materials to a chlorine content of up to 71% has been achieved by electrolysis methods, electric discharge and photo-chlorination. Products of intermediate chlorine content have been prepared, and some of these have extremely interesting olefinic functions.

At the University of Bombay, India, sulfated monoglycerides of lauric, myristic, palmitic and stearic acid were prepared in a pure state. Surface and interfacial tension, foam height,  $\text{Ca}^{++}$  stability, and emulsifying and lime soap dispersing properties were measured and compared with sodium dodecyl sulfate. Tallow derivatives had the best  $\text{Ca}^{++}$  stability, lime-soap-dispersion and emulsifying properties.



3. Lubricant investigations. The cyanoethylation of hydroxylated fatty esters has been achieved. Using excess acrylonitrile as solvent has led to yields in the 90-95% range and considerable progress has been made in converting the resulting  $\beta$ -cyanoethoxy ethers to  $\beta$ -carboxamidoethoxy ethers without significant losses due to ether cleavage. Two new types of materials derivable from fats and oils have been isolated, dihydroxytetrahydrofuran derivatives from diepoxides and hydroxyamide from aziridine. Since the internal aziridines react with a variety of carboxylic acids (pelargonic, stearic, benzoic and para-methoxy-benzoic) to form hydroxyamides, exploitation of this reaction in lubricant applications will be investigated.

Preparation and evaluation of larger quantities of pure  $\alpha$ -branched fatty acid esters for use as lubricants show that these compounds are equal to or superior to most other organic materials in viscosity, viscosity index, thermal stability and hydrolytic stability. In the wear test they show good results when used alone but are not susceptible to substantial improvement by known additives. Several new esters were prepared including the methyl ester of "isostearic acid." A commercial product consisting of a mixture of numerous branched acids was added to 1-decene to prepare a product with potentially useful lubricant properties.

4. Exploratory reactions investigations. The enol ester, isopropenyl stearate, is effective in stearoylations because it is a source of latent hexadecylketene, the actual acylating agent. Stearoketene dimer (2,4-dihexadecylcyclobutane 1,3-dione) has been obtained pure in high yield. The generation of stearoketene in the presence of hydroxylamine gave the previously unknown distearoyl and tristearoyl dihydroxamic and trihydroxyamic acids. The ketenes are capable of attacking amides and hydroxylamines and, in general, materials with active hydrogen functions.

The reaction of aroyl and diacyl peroxides with iodine in various solvents appears to be a new class of reaction. Thus, benzoyl peroxide and aliphatic acids and iodine react to give alkyl iodide derived from the aliphatic acids. This reaction offers a valuable alternative to the use of expensive silver-salt preparations for the preparation of alkyl iodides.

In grant research at the Hormel Institute of the University of Minnesota, progress has been made in techniques applied to the destructive distillation of ozonides. It is possible to select the operating conditions for a centrifugal type molecular still so as to either distill the ozonides of fatty esters for purification purposes or to thermally decompose the ozonides to obtain aldehydes and aldehydates in high yield.

At the Institut des Corps Gras, Paris, a detailed study has been completed of the volatile and nonvolatile products of the air oxidation of oleic acid at 40° and 20°C. Volatile organic materials include bis-(1-hydroxyheptyl) peroxide, saturated straight chain aldehydes (C<sub>6</sub> - C<sub>10</sub>) and propionic, acetic and formic acids. The grantee concludes that these compounds, as well as CO<sub>2</sub> and part of the water, arise from the splitting of the oleic acid hydroperoxide to give decanol and nonanol. Further stepwise degradation of the



aldehydes follow. The nonvolatile portion consisted of oxymonomers and oxypolymers.

In a study of the autoxidation of methyl erucate at Technical University, Gdansk, Poland, mathematical equations for change in double bond (as measured by iodine values) and change in hydroperoxide content as a function of time have been devised. The data indicate the presence of an undefined, initial catalyzing substance and that the reaction follows a three-step kinetic scheme from methyl erucate to peroxide decomposition products proceeding through an activated complex at each step.

Allylically brominated oleic acid has been converted into allylically hydroxylated unsaturated acids in good yields in a process employing milk alkali salts. In this research at Universite d'Aix, Marseille, France, selenium dioxide has also been used to produce allylically hydroxylated products in fair yields, while mercuric acetate gives nearly exclusively monohydroxy-allylic derivatives.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition, Physical Properties and Structure

- Andera, M. J., Anderson, L. V., Doughtie, R. T., Jr., Fink, K. H., Herb, S. F., Koester, W. H., Kuiken, K. A., and Miller, W. J. 1966. 48th Annual report of the Smalley committee 1965-1966. J. Am. Oil Chemists Soc., 43, pp. 287A, 292A.
- Barford, R. A., Luddy, F. E., and Magidman, P. 1966. The hydrolysis of long chain trisaturated triglycerides by pancreatic lipase. Lipids, 1, p. 287.
- Boucher, E. A., Grinchuk, T. M., and Zettlemoyer, A. C. 1967. Measurement of surface tensions of surfactant solutions. J. Colloid Interface Sci., 23, pp. 600-603.
- Cavanaugh, J. R., 1967. The rotational isomerism of phenylalanine by nuclear magnetic resonance. J. Am. Chem. Soc., 89, pp. 1558-1564.
- Eddy, C. R., and Showell, J. S. 1966. Uses of digital computers in theoretical analytical chemistry. I. Separation of a composite infrared band into two component peaks. J. Am. Oil Chemists Soc., 43, pp. 643-646.
- Eddy, C. R., and Showell, J. S. 1966. Uses of digital computers in theoretical analytical chemistry. II. Simulation of the passage of a compound through a countercurrent distribution apparatus. J. Am. Oil Chemists Soc., 43, pp. 647-648.
- Herb, S. F., Magidman, P., and Riemenschneider, R. W. 1967. Observations on response factors for thermal conductivity detectors in GLC analysis of fatty acid methyl esters. J. Am. Oil Chemists Soc., 44, pp. 32-36.
- Jensen, R. G., Marks, T. A., Sampugna, J., Quinn, J. G., and Carpenter, D. L. 1966. Purification of triglycerides with an alumina column. Lipids, 1, pp. 451-452.
- Lutz, D. A. 1967. Crystal data for N-n-butyloctadecanamide. Acta Cryst., 22, p. 154.
- Lutz, D. A., Eddy, C. R., and Hunter, J. J. 1967. X-ray diffraction study of some normal alkyl esters of long-chain acids. Lipids, 2, pp. 204-207.
- Nieschlag, H. J., Tallent, W. H., Wolff, I. A., Palm, W. E., and Witnauer, L. P. 1967. Mixed esters of brassylic acid as plasticizers for poly (vinyl chloride). Polymer Eng. Sci., 7, pp. 51-54.

- O'Connor, R. T., Allen, R. R., Chipault, J. R., Herb, S. F., and Hoerr, C.W. 1966. Report of the instrumental techniques committee, AOCS, 1964-1965. J. Am. Oil Chemists Soc., 43, pp. 10A, 12A, 34A.
- Purcell, J. M., Morris, S. G., and Susi, H. 1966. Proton magnetic resonance spectra of unsaturated fatty acids. Anal. Chem. 38, pp. 588-592.
- Riser, G. R., and Palm, W. E. 1967. A method for determining compatibility parameters of plasticizers for use in PVC through use of torsional modulus. Polymer Eng. Sci., 7, pp. 110-114.
- Sax, M., and McMullan, R. K. 1967. The crystal structure of dibenzoyl peroxide and the dihedral angle in covalent peroxides. Acta Cryst., 22, pp. 281-288.
- Scherr, H. J., 1967. Phototransducer for measuring relatively small angles. J. Sci. Instr., 44, pp. 479-481.
- Scherr, H. J., and Witnauer, L. P. 1967. The application of a capillary extrusion rheometer to the determination of the flow characteristics of lard. J. Am. Oil Chemists Soc., 44, pp. 275-280.
- Susi, H., and Jahn, A. S. 1966. Infrared anisotropy and structure of crystalline methyl stearate and methyl octadecyl sulfonate. J. Am. Oil Chemists Soc., 43, pp. 579-582.

#### Chemical and Physical Investigations to Improve Products

- Borka, L., and Privett, O. S. 1966. An electrostatic precipitator for preparative gas-liquid chromatography. Lipids, 1, pp. 104-106.
- Cordon, T. C. 1966. Synthetic detergents: some aspects of their relation to agriculture. Soil Sci., 102, pp. 1-17.
- Gadgoli, H. G., and Kane, J. G. 1966. Preparation of 1-monoglycerides by interesterification. Indian J. Chem., 4, pp. 289-290.
- Jordan, E. F. Jr., Monroe, H. A., Artymyshyn, B., and Wrigley, A. N. 1966. Intrinsic viscosity -- number average molecular weight relationship for poly(n-Octadecyl Acrylate) and poly(N-n-Octadecyl Acrylamide), J. Am. Oil Chemists Soc., 43, pp. 563-567.
- Jordan, E. F. Jr., Riser, G. R., Parker, W. E., and Wrigley, A. N. 1966. Mechanical properties and transition temperature for copolymers of N-n-alkylacrylamides and acrylonitrile, J. Polymer Sci., A-2, 4, pp. 975-996.
- Lai, R., Naudet, M., and Ucciani, E. 1966. The oxo synthesis in lipochemistry - preliminary study on the hydroformylation of methyl oleate. Rev. Franc. Corps Gras, 13, pp. 737-745.

- Maerker, G., Haeberer, E. T., and Herb, S. F. 1966. Epoxidation of methyl linoleate. II. The two isomers of methyl 9,10:12,13-diepoxysearate. J. Am. Oil Chemists Soc., 43, pp. 505-508.
- Maurer, E. W., Stirton, A. J., and Weil, J. K. 1966. Octadecylsulfuric acid. Biochem. Prep., 11, pp. 96-100.
- Micich, T. J., Diamond, E. A., Bistline, R. G. Jr., Stirton, A. J., and Ault, W. C. 1966. Sulfonated branched chain fatty acids and esters. J. Am. Oil Chemists Soc., 43, pp. 539-541.
- Riemenschneider, R. W., Zell, T. E., and Scott, W. E. 1966. Synthesis of methyl, propyl, butyl, isobutyl, hexyl, cyclohexyl, octyl, and 2-ethyl-hexyl esters of epoxyoleic acid. J. Am. Oil Chemists Soc., 43, pp.456-457.
- Rothman, E. S. 1967. Enol esters. VI. Hexadecylketene lactone dimer and di- and tristearoylhydroxamic acids. J. Org. Chem., 32, p. 1683-1684.
- Saggese, E. J., Scholnick, F., Zubillaga, M., Ault, W. C., and Wrigley, A. N. 1967. Urethane foams from animal fats. III. Oxypropylated dihydroxystearic acids in rigid foams. J. Am. Oil Chemists Soc., 44, pp. 43-45.
- Scholnick, F., Monroe, H. A. Jr., Saggese, E. J., and Wrigley, A. N. 1967. Urethane foams from animal fats. II. Reaction of propylene oxide with fatty acids. J. Am. Oil Chemists Soc., 44, pp. 40-42.
- Smith, F. D., Stirton, A. J., and Nunez-Ponzoa, M. V. 1966. Isomeric linear phenylalkanes and sodium alkylbenzenesulfonates. J. Am. Oil Chemists Soc., 43, pp. 501-504.
- Stirton, A. J., Bistline, R. G. Jr., Leardi, E. B. and Nunez-Ponzoa, M. V. 1965. Tallow-based detergent formulations: mixtures of alcohol sulfates, salts of  $\alpha$ -sulfo acids and esters, and soap. J. Am. Oil Chemists Soc., 44, pp. 99-102.
- Tubul-Peretz, A., Naudet, M., and Ucciani, E. 1966. Sur l'obtention de derives hydroxyles allyliques des chaines grasses monoinsaturees 11. oxydation par l'anhydride selenieux. Rev. Franc. Corps Gras, 13, pp.155-164.
- Tubul-Peretz, A., Ucciani, E., and Naudet, M. 1966. Sur la structure des produits formes au cours de l'action de  $\text{SeO}_2$  sur l'acide oleique. Bull. Soc. Chim. France, no. 7, pp. 2331-2336.
- Weil, J. K., Stirton, A. J., and Barr, E. A. 1966. Oxypropylation of fatty alcohols, and the sulfation products. J. Am. Oil Chemists Soc., 43, pp. 157-170.
- Maurer, E. W., Stirton, A. J., and Weil, J. K. December 13, 1966. Emulsion containing long chain alkyl sulfuric acids. U. S. Patent 3,291,750.



Maurer, E. W., Stirton, A. J., and Weil, J. K. February 21, 1967. Method for preparing substantially pure amino acid salts of alkyl sulfuric acids. U. S. Patent 3,305,578.

Port, W. S., and Ostapiak, D. M. February 21, 1967. Non-terminal epoxy phenolic ester monomers and resins thereof. U. S. Patent 3,305,564.

Roe, E. T., Konen, D. A., and Swern, D. March 7, 1967. Chain substituted higher fatty acids. U. S. Patent 3,308,140.

Rothman, E. S. March 7, 1967. Preparation of novel polymers. U. S. Patent 3,308,100.

Scholnick, F., Monroe, H. A. Jr., Wrigley, A. N., and Ault, W. C. June 6, 1967. Resins from hydroxyphenylstearic acid. U. S. Patent 3,324,059.

Weil, J. K., Stirton, A. J., Smith, F. D., and Bistline, R. G. Jr., January 10, 1967. Process for preparing a detergent composition containing sodium alkanesulfonate and soap. U. S. Patent 3,297,579.

#### AREA NO. 4. HIDES AND LEATHER UTILIZATION

Problem. To maintain the utilization of animal hides and skins at a profitable level there is need to find new products and processes to provide outlets for about 13 million cattlehides that are now available in excess of domestic needs. The foreign markets that currently absorb these surplus hides are also threatened by the increased hide production and decreased per capita use of leather (the principal outlet for hides) that have dislocated U.S. markets and caused prices to drop so precipitously in the last 10 years. To meet this problem there is need for upgrading the quality of raw hides and skins, for reducing the costs of producing leather, and for developing new and nonconventional products from collagen. To achieve these objectives research is needed to develop improved curing processes and agents, more effective control measures for (ante mortem) defects such as grubs, brands and parasite damage, and improved methods of take-off. Fundamental research is needed on the composition of hides to provide basic information on the chemical, physical and physical-chemical properties and reactions of collagen and other hide components for use in studies on chemical modification and on the development of new and improved products and processes. Development of new, more rapid and economic processes for curing, handling, unhairing and tanning hides is needed to reduce the cost of producing leather. There is also need for research on the chemical modification of hide proteins to develop leather products with such improved "built-in" properties as increased resistance to wear, scuffing and deterioration from perspiration, enhanced washability, dry-cleanability and improved dyeability. There is also need for research on the physical and chemical properties of collagen to obtain information for use in dispersing and regenerating the fibrous structure without degrading its unique properties for developing nonconventional products that will provide new outlets and markets for hide proteins, with special reference to the field of edible products.

#### USDA AND COOPERATIVE PROGRAM

The department is conducting a broad program of basic and applied research on hides, skins and leather at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out; this involves physicists, chemists, biochemists, microbiologists and leather technologists.

The Federal scientific effort devoted to the over-all program totals 27.2 scientist man-years, including contract and grant research equivalent to 2.1 scientist man-years per year, applied as follows:

(a) Research on chemical composition, physical properties and structure of hides and leather involves 10.7 scientist man-years at Wyndmoor. One line of investigations is concerned with the isolation of collagen and other hide components and with basic research on the chemistry of collagen. This research is supplemented by (1) a grant (0.6 scientist man-year) at Northwestern University School of Medicine, Chicago, to apply physical chemistry

techniques to protein solvent interactions of collagen and gelatin, (2) a contract (0.6 scientist man-year) at the same institution to study relation of high molecular weight gelatin to collagen, (3) contract research involving 0.5 scientist man-year at Midwest Research Institute, Kansas City, Missouri, to study the dispersion of collagen, and (4) a PL-480 grant at the University of Turku, Finland, (3 years, 1965-1968) for basic investigations on the structure, biosynthesis and maturation of collagen.

Other investigations at Wyndmoor are concerned with the relation of hide composition and structure to leather properties. Additional research is in progress at the Central Leather Research Institute, Madras, India, under PL-480 grants on (1) the hydrothermal shrinkage of collagen and leather, (3 years, 1964-1967) and (2) comfort properties of shoe leather (5 years, 1964-1969). The contract research at the University of Cincinnati, Cincinnati, Ohio, on the abnormalities of leather characterized by a depleted, mushy texture was completed during the reporting year (0.4 scientist man-year).

(b) Chemical and physical investigations to improve products involves 8.4 scientist man-years at Wyndmoor. This research is concerned primarily with the investigation of chemical modifications of hides prior to and during tanning operations to provide improved leather.

Research sponsored by the Department under PL-480 grants is in progress at Central Leather Research Institute, Madras, India, on (1) polyphenolic tanning compounds (5 years, 1962-1967) and (2) preparation and determination of physico-chemical properties of polypeptidyl derivatives of collagen (5 years, 1966-1971), and at the British Leather Manufacturers Research Association, Surrey, England, to investigate chemically reactive compounds for improving leather stability (5 years, 1963-1968).

(c) Technology - process and products development involves 6.0 scientist man-years at Wyndmoor. Research includes development of new tanning processes for hides and skins to provide products of superior durability and development of regenerated collagen products.

Additional research under PL-480 grants is in progress at Central Leather Research Institute, Madras, India, on (1) relation of hide quality to tanning rate (5 years, 1962-1967), (2) radioactive tracer study of mineral tanning (5 years, 1965-1970), and (3) rapid tannage of sole leather (5 years, 1965-1970); and at the Leather Research Institute, T.N.O., Waalwijk, Holland, on kinetics of chrome tanning (4 years, 1964-1968).

#### PROGRAM OF STATE EXPERIMENT STATIONS

State stations reported no research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure.

1. Protein constituents and collagen. Nuclear magnetic resonance studies of the phenylalanine cation and anion in aqueous solution suggest that the relative potential energies of three rotamers of this substance are dependent on temperature and concentration. The dependence correlates with the dielectric constant of the aqueous solutions. Application of infrared spectroscopy to the characteristic vibrational frequencies and other physical constants for different configurations of proteins in deuterium oxide solution shows that the fine structure of certain infrared bands permits distinguishing between the  $\alpha$ -helical extended and solvated random configuration for these proteins.

The autoclaving conditions which almost completely dissolve collagen solubilizes only 12% of epoxy resin-treated collagen. Under these conditions the average peptide length of the solubilized material was 20 units for collagen and 6 units for the epoxy-treated collagen. Controlled experiments with standard amino acid mixtures treated with aldehydes showed that aldehydes caused total disappearance of the tyrosine of collagen. It was also found that even small amounts of decolorizing carbon would cause the complete disappearance of tyrosine, phenylalanine and methionine and a partial disappearance of leucine, isoleucine, lysine and arginine from collagen hydrolyzates. The interference of amino acid analysis by aldehydes and decolorizing carbon is not widely known or appreciated and this possibility should be considered in interpreting results from experiments known or suspected of involving these treatments.

Contract research at the Midwest Research Institute, Kansas City, Missouri, showed that dispersed collagen sols can be obtained without the use of acids by liming hides for six weeks or longer. Relatively concentrated dispersions have been prepared from the hydrochloride of collagen. Under acid conditions the sodium chloride concentration considerably affects the dispersibility of the collagen hydrochloride. Fibers and films have been prepared from these collagen dispersions. In grant research at the Northwestern University School of Medicine, Chicago, gelatin was employed to investigate solvent-solvent and solvent-peptide interactions in the solvent pairs formic acid-methyl acetamide, formic acid-methyl formamide, formic acid-dimethyl sulfoxide and formic acid-acetonitrile. The interaction potential between the solvent system and solubilized collagen was examined for the solvent pairs formic acid dimethyl-formamide, formic acid-dimethylsulfoxide, formic acid-water and formic acid-acetonitrile. These data support the idea that the solubilized collagen molecule is stabilized by one definite type of intramolecular force. Analysis of mutarotation data showed that water has no specific effect on the mutarotation of poly-L-proline and has a large effect on stabilizing poly-L-hydroxyproline. The effect of water suggests that this force is either hydrogen bonding or hydrophobic bonding.



Two new and significant findings have resulted from research under a PL-480 grant at the University of Turku, Finland. The requirement of calcium for the maturation of collagen may have significance to our own studies on dispersibility of mature collagen. The procedure for the isolation of large collagen fragments after partial degradation with pepsin will aid materially in future studies of collagen structures.

2. Hides and leather. It has been conclusively established that a defect known in the trade as "pulpy butts" which produces weak leather is due to an abnormal vertical alignment of fibers and usually a high fat content in the hides themselves. It occurs mainly in a localized area on the back of a small percent of thick, heavy Hereford hides and appears to be of a genetic origin. The defect "cockle" in sheepskin, in which nodules in defective skins assume the form of hard ridges in the finished leather, makes such areas unfit for either grain or suede types of leather. The problem is seasonal, occurring only during the colder months. Early in the season the lesions are small but gradually become more numerous and more elevated, tend to be aligned in rows and can cover almost the entire skin. It is impossible to detect the early lesions in unprocessed skin even after shaving off the wool. The application of differential thermal analysis and dynamic mechanical testing showed that the shrinkage temperatures of leathers stored at 23°C. and 50% relative humidity decreased with storage time. Sole leather exhibited most resistance to change in shrinkage temperature while chrome-tanned vegetable retanned leathers showed the greatest variation in shrinkage temperature under the various conditioning treatments used.

The automated equipment used to solubilize calfskin collagen has resulted in solubilization of 7% of the calfskin corium used as the source of collagen. In previous runs with manual adjustment of solubilizing condition the yields of solubilized collagen ran consistently below 1%. Citrate bound to the corium after exhaustive solubilization showed eight citrate residues per un-solubilized collagen molecule, the same as previously observed for reconstituted collagen. The corium residue from the automated process yielded no further solubilized collagen under treatment of a variety of other solubilizing agents. Other investigators have reported the occurrence of several species of collagen, each of which is amenable to a different solubilizing agent, but these experiments show that under optimum conditions a single solubilizing agent is adequate. The citrate determinations indicate similarity between reconstituted collagen and the corium after complete solubilization.

Studies under a PL-480 grant at the Central Leather Research Institute, Madras, India, indicate that various factors influence the hydrothermal shrinkage of animal hides. These include: (a) swelling, (b) deposition, (c) fiber structure, (d) drying, and (e) chemical binding. The behavior of skin or leather is the sum total of the behavior of the collagen fibers. The shrinkage properties vary with the treatments given the collagen.

Under another PL-480 grant at the Central Leather Research Institute, studies have continued on the various effects of moisture upon foot comfort. The experiments of the previous year involved water absorption measurements on

leather samples under laboratory controls. These techniques have now been applied to leather samples placed at twelve locations both inside and outside the sock under actual wear conditions. Since these samples were equilibrated at 65% relative humidity before the experiments and only increases in moisture content were detected, the micro climate within all regions of the shoe must be above 65% relative humidity. The toe parts of the shoe show the greatest increases in moisture uptake of the samples. When water vapor impermeable materials are placed between the samples and the outside of the shoe, there is an increase in the moisture uptake proving that transpiration of moisture through the shoe is effective in reducing the humidity or moisture conditions within the shoe. It has been determined that both the left and right foot have comparable moisture relationships; and, therefore, one foot can be used as a control for an experiment performed on the other.

## B. Chemical and Physical Investigations to Improve Products.

1. Mannich reactions. The reaction of malonic acid and formaldehyde and the hide protein results in a new substrate with enhanced binding power for chrome and other mineral tanning agents. The enhanced binding power is a result of the introduction of additional carboxyl groups on to the collagen. Amino acid analysis indicated that chemical modification was accomplished and that generally the lysine, hydroxylysine and histidine residues were involved. The products when retanned with chrome showed greater stability to aging and greater resistance to deterioration from hot soap solution than conventional chrome-tanned leather. The Mannich reaction thus appears to be an easy method for modifying hide substance.

2. Other chemical modifications. Amino acid analyses showed that approximately two-thirds of the lysine originally present in sheepskin disappeared after tannage with glutaraldehyde. In addition some hydroxylysine also appeared to be lost. No other amino acids appeared to be involved in the fixation of glutaraldehyde. A series of drycleaning tests on suede garment leathers showed that glutaraldehyde retannage and a fat liquor based on Deriphats ( $\text{RNHCH}_2\text{CH}_2\text{CO}_2\text{H}$  and derivatives) gave products much more retentive as a fat liquor than commercial fat liquors. Three drycleaning agents were used--Zalclene, Stoddard solvent and perchloroethylene. In studies of the chemical nature of the product produced by tanning collagen with glutaraldehyde it was found that tests with 3-methyl-2-benzothiazolone hydrazone hydrochloride (NBTH reagent) show that collagen tanned with glutaraldehyde has free aldehyde groups.

Combination tannages with resorcinol in conjunction with methylolated products gave best results with the combination of resorcinol and dimethylol-propylene-urea. The product was a white soft leather. Tannage with dimethylol-urea followed by zirconium or chrome gave a soft leather resistant to artificial perspiration. A preliminary test on the dimethylol-urea tanned leather indicates good resistance to deterioration by acidic peroxide (a standard test for bookbinding leathers).

In research on polyphenolic tanning compounds under a PL-480 grant at the Central Leather Research Institute, Madras, India, grantees have obtained a greater knowledge of the composition and properties of babul, dhawa and goran tanning materials. Work is concentrated on extraction of the tanning materials and their complexing with chrome as tanning agents.

Research on the modification of collagen with aldehydes under a PL-480 grant with the British Leather Manufacturers Research Association, Surrey, England, was continued. The reactivity of different aldehydes was systematically explored. Characterization of the types of products formed has provided information on the possible mode of action and on the structure of the addition products. The interpretations are tentative, but are a basis for more definitive experiments.

3. Pretannage investigations. Aqueous butyl carbitol (approx. 15% in carbitol), used to dehydrate animal hides and skins, inhibits both bacterial and mold growth. The effectiveness of dehydration of animal hides and skins by this method can be evaluated by measurement of the apparent density of the dehydrated product. The method is promising and appears to reflect the nature of the fiber separation. Dehydrated goat skins when processed commercially gave leather considered equal to goat skins produced in the usual way. As raw hides and skins are perishable, dehydration, if it can be accomplished economically, becomes an attractive method for preserving and stabilizing hides.

Contract research at the University of Cincinnati indicates that the extraction of fresh hides with 1% sodium chloride solution prior to brine curing has only slight effect on the characteristics of the unfinished leather. It is concluded that it is not economically justifiable to extract the non-collagenous proteins from cattle hides prior to curing. It is also concluded that the water wash now used by some packers prior to fleshing and demanuring will not harm the quality of the hides and may be beneficial. This is the final report of a previously completed contract.

#### C. Technology - Process and Product Development.

1. Glutaraldehyde tannage. A large-scale tannery pack of shearlings made with glutaraldehyde and an appreciably higher amount of basic chromium sulfate yielded medical pads with an increased degree of hydrothermal stability which provides a greater margin of safety in laundering. After six months use in Philadelphia hospitals these shearlings are washing and wearing well.

Bookbinding leather made by tanning sheepskin with glutaraldehyde without the usual 5% potassium lactate buffer yielded excellent (but not perfect) resistance to the standard test for bookbinding leather which uses a combination of sulfuric acid and hydrogen peroxide as the test reagent. Since bookbinding leather is an imported commodity, oxidation-resistant leather might provide a market for domestic hides and skins.

2. Chrome tannage. The exchange of sulfate tagged with  $S^{35}$  atoms shows slow



and fast exchange rates which indicate more than one chromium complex is present in chromium tanning materials. The presence of OH groups in the complex is responsible for the fast exchange rate. Fractionation of these complexes on Dowex 50 ion exchange columns demonstrated complexes with 1, 2 and 3 positive charges. The complexes with 2 and 3 charges showed no exchange between bound and free sulfate groups. Exchanges of chromium complexes on carboxylic and sulfonic type ion exchange resins show that some species of complex which bind readily to carboxylic type resins do not bind to sulfonic type resins. This supports the present belief that the chromium tanning materials are bound chiefly to the carboxylic groups of proteins. This research was conducted under a PL-480 grant at the Central Leather Research Institute, Madras, India.

In the research under a PL-480 grant at the Lederinstitut, T.N.O., Waalwijk, Holland, the influence of temperature, basicity, and concentration of chrome tanning agents were studied simultaneously. Basicity effects were more pronounced as temperature increased, but only for the first period of tanning.

As masking agents, sodium salts were effective in this order: butyrate, formate, propionate and acetate. They stabilized the chrome complex and reduced the amount of chrome fixed by a moderate amount at pH's of 3.0 - 4.0. A small amount of oxalic acid was observed to increase the fixation of chrome.

An accurate method of measuring ionic sulfate in tanned leather was developed.

3. Other tanning research. Research at the Central Leather Research Institute, Madras, India, is proceeding under PL-480 grants to study (a) the rapid tannage of sole leather and (b) relation of hide quality to tanning rate. In the former, Indian tanning materials were applied in rapid tanning of heavy leather in small-scale experiments. Bringing the pelt to its isoelectric point before vegetable tanning increased rate of penetration without adverse effect on the fixation of tans and leather properties. Osmotic process and the chrome pretreatment method gave leathers of good yield and good physical properties. Tanning at higher temperatures gave the quickest penetration, but the feel and the physical properties of the resultant leather were not very satisfactory.

In the latter, many of the variables affecting hide quality and leather properties have been studied. A significant contribution has been the development of a laboratory test method for the determination of hide quality. This is based on the measurement of hydroxyproline in the soak waters from the hides. Hydroxyproline being an amino acid characteristic of collagen, its presence correlates with conditions of hide history that lead to degradation of hide substance. Changes in the noncollagenous proteins in hides caused by different curing procedures were evaluated. The history of the hide (slaughtered or fallen) was also considered. Procedures for controlled tanning and for determining the extent of tanning were standardized.



4. Regenerated collagen products. Since limed hides are commercially available and yield dispersions with the same properties as enzyme-unhaired hides, the latter are no longer under study to produce collagen dispersions. Two routes to a collagen dispersion useful in food applications have been applied to lime unhaired hides, (1) a cold acid process and (2) a warm acid process. Films obtained from the warm acid process did not dissolve in warm water and shrank like native collagen rather than dissolving like gelatin. Reducing sugars are being evaluated as cross-linking agents in such films. Cross-linked films are pliable, elastic, easily stripped from a surface and resist disintegration at 170°C. in the smokehouse.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition, Physical Properties and Structure

- Bitcover, E. H., and Mellon, E. F. 1966. Distribution of lipid classes with respect to area and stratigraphic location in a steerhide. J. Am. Leather Chemists Assoc., 60, pp. 338-350.
- Bitcover, E. H. and Penrose, S. 1967. Technic for cleaning thin-layer chromatographic plates. Chemist-Analyst, 56, p. 32.
- Everett, A. L., Willard, H. J. and Windus, W. 1967. Microscopic study of leather defects. II. Inherent vertical fiber structure in side leather. J. Am. Leather Chemists Assoc., 62, pp. 25-44.
- Filachione, E. M., Korn, A. H. and Ard, J. S. 1967. The ultraviolet absorption of protein-bound glutaraldehyde. J. Am. Leather Chemists Assoc., 62, pp. 450-453.
- Hollmen, T., and Kulonen, E. 1966. Separation of the components in collagen by preparative starch-gel electrophoresis. Anal. Biochem., 14, pp. 455-466.
- Hollmen, T., and Kulonen, E. 1966. Combination of temperature gradient with gel electrophoresis and its applications to analysis of collagen-gelatin transition. J. Chromatog., 21, pp. 454-459.
- Jahn, A. S., and Witnauer, L. P. 1967. Water binding in hide materials. J. Am. Leather Chemists Assoc., 62, pp. 334-345.
- Kahn, L. D., and Witnauer, L. P. 1966. The viscometric behavior of solubilized calfskin collagen at low rates of shear. J. Biol. Chem., 241, pp. 1784-1789.
- Kahn, L. D., and Witnauer, L. P. 1967. A viscometric study of solubilized calfskin collagen using the Zimm-Crothers viscometer. Biochim. Biophys. Acta, 133, pp. 184-185.
- Kumeto, A., Kari, A., Hollmen, T., and Kulonen, E. 1966. On the intracellular synthesis of collagen. Ann. Med. Exp. Biol. Fenniae, 44, pp. 58-62.
- Lampiaho, K., Juva, K., and Kulonen, E. 1965. Incorporation of amino acids into fractions of insoluble collagen. Ann. Med. Exp. Biol. Fenniae, 43, pp. 254-256.
- Mellon, E. F., Gruber, H. A., and Bitcover, E. H. 1967. Differences in amino acid content between calfskin and ligament elastins. J. Am. Leather Chemists Assoc. 62, pp. 90-96.

- Mikkonen, L., Lampiaho, K., and Kulonen, E. 1966. Effect of thyroid hormones, somatotrophin, insulin and corticosteroids on synthesis of collagen in granulation tissue both in vivo and in vitro. *Acta Endocrinol.*, 51, pp. 23-31.
- Palm, W. E., Bloom, F. W., and Witnauer, L. P. 1966. Nondestructive dynamic compression measurements of full sides of leather. *J. Am. Leather Chemists Assoc.*, 61, pp 222-234.
- Pikkarainen, J., and Kulonen, E. 1965. The fractionation of various gelatin preparations with ion-exchange resins. *Suomen Kemistilehti B*, 38, pp. 226-230.
- Purcell, A. W., Jahn, A. S., and Witnauer, L. P. 1966. Differential thermal analysis of the helix-coil transition in solubilized collagen. *J. Am. Leather Chemists Assoc.*, 61, pp. 273-274.
- Scherr, H. J. 1966. Measuring the amplitude of vibration of a reed. *Mater. Res. Std.*, 6, pp. 614-616.
- Susi, H., and Ard, J. S. 1966. Near infrared investigation of interamide hydrogen bonds in hydrogen-bonding solvents. *Arch. Biochem. Biophys.*, 117, pp. 147-153.
- Tancous, J. J., Schmitt, R., and Windus, W. 1967. The incidence and predictability of weakness and pulpiness in side upper leather. *J. Am. Leather Chemists Assoc.*, 62, pp. 4-24.
- Wiegand, C. J. W. December 20, 1966. Process of solubilizing native collagen by reacting said collagen with a stoichiometric amount of a mineral acid. U. S. Patent 3,293,273.

#### Chemical and Physical Investigations to Improve Products

- Bowes, J. H., and Cater, C. S. 1965. The reaction of glutaraldehyde with proteins and other biological materials. *J. Roy. Microscop. Soc.* 85, pp. 193-200.
- Feairheller, S. H., Taylor, M. M. and Filachione, E. M. 1967. Chemical modification of collagen by the Mannich reaction. *J. Am. Leather Chemists Assoc.*, 62, pp. 398-407.
- Feairheller, S. H., Taylor, M. M. and Filachione, E. M. 1967. The Mannich reaction with malonic acid and formaldehyde as a pretreatment for mineral tannages. *J. Am. Leather Chemists Assoc.*, 62, pp. 408-422.
- Hopkins, W. J., Luvisi, F. P., and Filachione, E. M. 1967. Influence of alkenyl succinic acid lubrication on water-repellent treatments for leather. *J. Am. Leather Chemists Assoc.*, 62, pp. 162-173.

- Luvisi, F. P., Hopkins, W. J., Naghski, J. and Filachione, E. M. 1966. Fatliquoring with alkenyl succinic acids. J. Am. Leather Chemists Assoc., 61, pp. 384-393.
- Luvisi, F. P., Hopkins, W. J., Naghski, J., and Filachione, E. M. 1966. Alkenyl succinic acid-silicone systems for water-resistant leathers. J. Am. Leather Chemists Assoc., 61, pp. 585-593.
- Santhanam, P. S., Ghosh, D., and Nayudamma, Y. 1965. Studies on the phenolic constituents of babul. I. Affinity studies. Leather Sci., 12, pp. 179-183.
- Viola, S. J., and Fein, M. L. 1966. Leather golf gloves with improved washability and perspiration resistance. Leather Mfr., 83, no. 8, pp. 16, 18, 20.
- Viola, S. J., Fein, M. L., and Naghski, J. 1966. Washability of glutaraldehyde-chrome-tanned garment and glove leathers. J. Am. Leather Chemists Assoc., 61, pp. 661-670.
- Fein, M. L., Harris, E. H. Jr., and Calhoun, R. R. March 7, 1967. Apparatus for determination of shrink-temperature of leather. U. S. Patent 3,307,815.
- Fein, M. L., Viola, S. J., and Filachione, E. M. January 24, 1967. Process for treating washable leather. U. S. Patent 3,300,338.
- Hopkins, W. J., Luvisi, F. P., and Filachione, E. M. December 20, 1966. Dehydration and preservation of animal hides and skins. U. S. Patent 3,292,271.
- Weaver, E. A., and Pollack, R. L. August 24, 1965. Self-demountable support. U. S. Patent 3,202,484.

#### Technology -- Process and Product Development

- Khanna, J. K., Nandy, S. C., Venkatachalam, P. S., and Nayudamma, Y. 1966. Studies on the qualities of sole leathers from slaughtered and fallen buffalo hides. Leather sci., 7, pp. 191-197.
- Naghski, J. 1966. Government services and direct cooperation with and for industry. Leather Mfr., 83, no. 11, pp. 64-67, 79-84.
- Windus, W. 1966. The tanning of hides and skins. Encyclopedia of Chemistry, Reinhold Pub. Corp., New York, New York, pp. 1034-1036.



## AREA NO. 5. POTATO UTILIZATION - FOOD

Problem. The potato industry, faced with a continuing decline in the consumption of fresh potatoes, has turned to and is becoming more and more dependent upon the development of new and improved processed products to maintain markets and avoid recurring economic disasters. Crop perishability, fluctuations in supply, and inelasticity of demand, result in wide price swings with even slight surpluses. Depressive lows are moderated by advance contracting by processors prior to harvest in producing areas having a substantial processing industry. However, in many processing areas, processing has not yet been developed and vulnerability still exists and is exaggerated by the growing competition of processed potato and other vegetable food products. If processing is to expand rapidly enough to offset progressive decline in fresh potato consumption, a continuing improvement in currently produced products and development of new products is clearly required.

Lack of adequate knowledge concerning the chemical constituents, physical properties, and enzyme systems in potatoes is limiting development of new and improved products and processing methods. Basic research on composition is needed to provide fundamental information on which an applied research program can be systematically and effectively built. Recently developed techniques make possible the isolation, characterization, and analysis of constituents responsible for flavor, color, odor, and texture of many processed food products which were not available to research in the past. Application of such techniques to potatoes and potato products should make possible the improvement of the quality of present products, both freshly processed and following storage, and provide a basis for technological and engineering studies in new product development.

### USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program of basic and applied chemical and engineering research on studies related to processing. The work of the EURDD, involving the services of chemists, biochemists, food technologists and chemical engineers at Wyndmoor, Pennsylvania, and East Grand Forks, Minnesota, is conducted in cooperation with several Agricultural Experiment Stations which supply potatoes of known cultural history and with the marketing research facilities of the Department. The chemical research program includes: isolation and characterization of the amino acid-sugar intermediate compounds responsible for the browning of chips and French-fried potatoes during processing; studies on lipids, which are believed to play an important role in the storage stability of processed potato products, particularly dehydrated products; isolation and characterization of the proteins, which are important from a nutritional aspect and from their possible involvement in textural and processing characteristics; elucidation of the causes of after-cooking discoloration and isolation and characterization of the pigment formed; methods of predicting textural characteristics of potatoes for French-fried potatoes. The Eastern Division's engineering and development research program seeks to

improve the quality, nutritive value and storage stability of dehydrated potato products and to develop more convenient types of dehydrated products, such as "instantized" pieces that rehydrate and cook quickly. The Red River Potato Processing Laboratory, East Grand Forks, Minnesota, has been established to conduct investigations relating variety and other raw material characteristics to quality of established forms of processed potatoes. This Laboratory is a cooperative undertaking of the Red River Valley Potato Growers Association, University of Minnesota, North Dakota State University, and the Agricultural Research Service.

The Federal scientific effort devoted to this area at Wyndmoor and East Grand Forks, Minnesota, totals 13.0 scientist man-years. Of this number, research on chemical composition and physical properties amounts to 8.2, research on color, texture and other quality factors amounts to 2.4, and research on technology-process and product development comprises 2.4 scientist man-years.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 7.5 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition and Physical Properties

1. Basic studies on potato lipids. Both the amount of unsaturated fatty acids (linoleic and linolenic) and the ratio of these to total fatty acid content start at a relatively high level during early growth and fall off to a low point near maturity. During storage there is only a slight rise in the percentage of unsaturated acids in potatoes. Processing to flakes and dice results in only slight changes in the fatty acid content and little oxidation of the unsaturated acids. It thus appears that a time close to maturity appears to be best for obtaining tubers of best quality for processing.

2. Potato proteins. Gel electrophoresis has demonstrated the presence of 20 bands in the soluble protein fraction of potatoes. One of these is a yellow band which is not an artifact. Separating the components of the soluble proteins is experimentally difficult. One large band detected by ultraviolet absorption techniques does not appear to be a protein when tested by the standard biuret-type reagent.

3. After-cooking discoloration. It has been verified that large potatoes darken more than smaller potatoes of the same lot. Of potatoes which darken, the stem-end is the location of greatest darkening and relative to the bud-end has the following characteristics: (1) low organic acid content; (2) low potassium content, or more probably, a high potassium to citric acid ratio; (3) high polyphenolic content; (4) high sodium content; and (6) low citric acid polyphenol ratio.

4. Reducing sugars and enzyme activity in stored potatoes. The levels of soluble sugars, invertase and invertase inhibitor in Kennebec potatoes of the 1966 crop were similar in many respects to the 1965 crop. Potatoes stored at 65°F. for three months did not develop enough reducing sugars to affect chipping quality. An excess of inhibitor was always present in these tubers. Tubers placed in storage at 40°F. developed high levels of reducing sugars in three weeks. During reconditioning the reducing sugars decreased to levels lower than those observed for the 1965 crop. The variety, 5899-1 (in North Dakota variety), a better chipper than Kennebec, showed a much higher level of excess inhibitor than Kennebec at harvest time. This variety reconditioned to lower levels of reducing sugar than Kennebec.

The inhibitor naturally present is a protein with a molecular weight of about 17,000 atomic mass units. It inhibits potato tuber invertase most effectively at the pH optimum for the enzyme (pH 4.5). Since both the invertase and the inhibitor are proteins, genetic control of these is a promising area for investigation.

#### B. Color, Texture and Other Quality Factors

1. Texture of French-fried potatoes. Progress has been made in developing an objective test for the quality of French-fried potatoes at the point of consumption. The use of liquid nitrogen for quick-freezing the pieces after frying and the use of a high-speed recorder which permits magnification of the force peaks has given reproducible results. The improved test measured differences in properties as the result of storage that could not be obtained by the use of a taste panel.

2. Pigments formed in potato chip frying. Use of filter paper "chips" prepared with known sugars and amino acids have permitted investigation of the reaction of these substances under conditions comparable to potato chip preparation. The nitrogen compounds included tyrosine, arginine, phenyl-alanine, tryptophan, histidine, ammonium sulfate, lysine and  $\gamma$ -aminobutyric acid. The sugars investigated include glucose, fructose and sucrose. Sucrose reacts with lysine at 103°C. much more slowly than with the other amino acids studied. Lysine reacts rapidly with glucose and fructose. The polymerization of the reaction products appears to be the cause of darkening in potato chips as heating continues.

Work on isolating the principal browning precursors is continuing.

#### C. Technology - Process and Product Development

1. Quick-cooking dehydrated potato products. Optimum conditions have been found for puffing 3/16-inch thick potato slices in the batch gun with superheated steam. The maximum charge size for slices was found to be 15 pounds as against 20 pounds for potato dice. Potato dice from Maine Katahdin potatoes of 18% solids are as good in every respect as those made from Idaho potatoes of 22% solids content. The Maine potatoes had less tendency to slough--a desirable attribute. Experiments in the continuous explosion-

puffing pilot plant showed that potato dice tended to clump. This problem was overcome by coating the partially dried pieces before puffing with powdered sodium silico aluminate (0.75% based on potato solids. This additive is rated GRAS). A feed rate of 675 pounds per hour at 18% moisture content, equivalent to about 590 pounds per hour of product at 6% moisture, has been achieved. However, it has not been yet possible to obtain continuously the high degree of puffing obtained with the batch gun. A problem still to be solved is the development of "off-flavors" in the puffing step. Studies have been begun, cooperatively between the Engineering and Development and Plant Products Laboratories, to identify substances causing these flavors.



PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

- Fitzpatrick, T. J. and Porter, W. L. 1966. Changes in the sugars and amino acids in chips made from fresh, stored and reconditioned potatoes. Am. Potato J., 43, pp. 238-248.
- Pressey, R. and Shaw, R. 1966. Effect of temperature on invertase, invertase inhibitor, and sugars in potato tubers. Plant Physiol., 41, pp. 1657-1661.
- Pressey, R. 1966. Separation and properties of potato invertase and invertase inhibitor. Arch. Biochem. Biophys., 113, pp. 667-674.
- Schwartz, J. H., Greenspun, R. B., and Porter, W. L. 1966. Chemical composition of potatoes. V. Further studies on the relationship of organic acid concentrations to specific gravity and storage time. Am. Potato J., 43, pp. 361-366.
- Zaehring, M. V., Reeves, R. M., Talley, E. A., Murphy, H. H., Dinkle, D. H., and Hyde, R. B. 1966. The estimation of total solids from specific gravity measurements. Potato Handbook, XI, pp. 46-48.

Color, Texture and Other Quality Factors

- Porter, W. L., and Ross, L. R. 1966. Factors affecting the textural quality of French fried potatoes. In Plant Science Symposium Proceedings. Camden, N. J. Campbell Institute for Agricultural Research, pp. 27-39.
- Ross, L. R. and Porter, W. L. 1966. Preliminary studies on application of objective tests to texture of French fried potatoes. Am. Potato J., 43, pp. 177-183.
- Shaw, R. 1966. Can pesticides affect potato quality? Valley Potato Grower, 19, no. 5, p. 5.

Technology -- Process and Product Development

- Eskew, R. K., Drazga, F. H., and Talley, F. B., 1966. The effect of nonfat milk solids on the storage properties of potato flakelets, U. S. Agricultural Research Service, ARS-73-52.
- Treadway, R. H. 1967. Non-food outlets for potatoes: starch and feed. Potato Handbook, XII, pp. 64-67.
- Treadway, R. H. 1967. Manufacture of potato starch. In R. L. Whistler and E. F. Paschall eds. "Starch: Chemistry & Technology," 2, pp. 87-101. New York Academic Press.

## AREA NO. 6. VEGETABLE UTILIZATION - FOOD

Problem. Vegetable growing occupies over 3 million acres, with a yearly farm value of a billion dollars. Utilization as processed rather than fresh vegetables provides a constant source of supply with less price fluctuation. Basic compositional research is needed to provide knowledge to constituents responsible for color, flavor and texture of vegetables and the changes these constituents undergo during processing, storage, and distribution. There is also need for application of these results to developmental research on new products and new and improved processing technology. Consumer preference is shifting to "convenience" foods. An even greater emphasis on quickly prepared foods is evident in modern military feeding where high bulk density, non-refrigerated, and rapidly rehydrating products are of primary importance.

### USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program employing chemists and chemical engineers in basic and applied research on vegetable processing and products. The Federal work is conducted at Wyndmoor, Pennsylvania. The scientific effort assigned to this area totals 3.5 scientist man-years and is currently engaged in research on technology-process and product development.

### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 28.7 scientist man-years is devoted to this area of research.

### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

#### A. Technology - Process and Product Development

1. Quick-cooking dehydrated vegetable pieces. Carrot dice have been successfully prepared in the continuous explosion-puffing pilot plant. The output of carrot pieces at about 25% moisture content has reached 1000 pounds per hour. At a moisture level of 23% an output rate of 935 pounds per hour was achieved. The 23% moisture content dice is a better product because it is free-flowing and nonsticky. California celery in one-half-inch slices, water blanched in alkaline solution (pH 8.2 - 8.6), dried to 35% moisture and puffed at 40 pounds per square inch gave a product of excellent color and texture. The dice rehydrated satisfactorily in four minutes, the color of the product is good, and the flavor satisfactory.

2. Dehydrated mushroom products. The through-circulation drying of dice and slices of cultivated Agaricus campestris mushrooms (recently termed Agaricus bisporus) yielded good results if the mushrooms were very fresh, if they were of the white variety and if low temperatures (90°F. or lower) were used either throughout the whole drying cycle or initially followed by drying at a higher temperature. The air-dried mushrooms lose initial flavor in approximately one to two months if the moisture content is 10% or higher. If dried to 6.5%

moisture they retain flavor at least six months. These studies suggest that it should be possible to make a good dried product in particulate form by controlled hot-air drying and that such a product should help growers meet competition from Taiwan.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Technology -- Process and Product Development

Komanowsky, M., Turkot, V. A., Talley, F. B., and Eskew, R. K. 1966. Drum-dried mushroom powder a new product. U. S. Agricultural Research Service, ARS-73-53.

Turkot, V. A., Sullivan, J. F., Cording, J., Jr., and Eskew, R. K. 1966. Explosive puffing enhances quick-cook qualities. Food Eng. 38, no. 12, pp. 77-79.

Heiland, W. January 24, 1967. Puffing gun. U. S. Patent 3,299,799.



## AREA NO. 7. DECIDUOUS FRUIT AND TREE NUT UTILIZATION - FOOD

Problem. Lack of knowledge of the nature and quantities of the various chemical constituents and enzyme systems present in fresh fruits, and of the changes these undergo during processing, is a limiting factor in research designed to develop new and improved products and processing techniques. Knowledge is required on the composition and physical structure of fruits and fruit products, with emphasis on substances responsible for color and flavor, vitamins, and other constituents important in determining consumer acceptance and nutritive value of the products. Composition should be studied in relation to variety, stage of maturity, and environmental conditions of growth; and to changes occurring between harvesting and processing, during processing, and in storage and distribution. Recently developed equipment and techniques have made it possible to isolate, separate, and identify constituents that could not have been handled previously. As basic information is developed, new processing techniques will be applied in the improvement of fruit products, and in more efficient utilization of by-products from fruit processing.

### USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists and chemical engineers engaged in both basic and applied research related to fruit processing and fruit products.

The Federal scientific effort devoted to research in this area totals 8.5 scientist man-years. Research on chemical composition and physical properties involves 0.6 scientist man-year, including effort equivalent to 0.2 scientist man-year under a grant at Temple University on the metabolism of red tart cherries. Effort on flavor research amounts to 1.1 scientist man-years, and research on color, texture and other quality factors involves 2 scientist man-years. Research on processing characteristics of Eastern pears will be conducted under a contract with Rutgers University, New Brunswick, New Jersey. Research on technology - process and product development involves 4.8 scientist man-years.

### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 38.5 scientist man-years is devoted to this area of research.

### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

#### A. Chemical Composition and Physical Properties

Contract research at the Maryland Agricultural Experiment Station, College Park, is now complete. This research showed that the calcium firming of apple slices appears to be due more to a complex of calcium with hemicellulose or cellulose rather than a calcium pectin combination. The pentosan-hexosan-

polyuronide complex, previously shown to be correlated with texture of canned apple slices, was found to be mainly  $\alpha$ - and  $\beta$ -D-xylose and  $\alpha$ - and  $\beta$ -L-arabinose plus small amounts of galactose, glucose and galacturonic acid. Stored apples contained small quantities of pectin esterase but no polygalacturonase. Autoradiography of apple slices treated with radioactive calcium is a useful technique for studying the calcium penetration and firming as effected by various processing methods. The firmness of canned slices was improved by vacuum treatment.

In grant research at Temple University, study of the metabolism of bruised and unbruised cherries by means of radioactive metabolites continued. Ripe cherries were subjected to bruising and injected with specific  $C^{14}$  labelled compounds. The rates of production of  $C^{14}O_2$  and total  $CO_2$  were determined. The fruit was then frozen for subsequent study of the various fractions developed during the research. Mature red tart cherries harvested with a portion of the stem attached were "fed" through the stem with three  $C^{14}$  labelled compounds. The fruit was then bruised and held for one hour or 24 hours. The various extracts and insoluble residues will be fractionated by suitable techniques to determine where  $C^{14}$  activity resides.

#### B. Flavor

Study of the composition of 150-fold Montmorency cherry essence showed the most volatile fraction to consist of acetaldehyde with smaller amounts of propanal, 2-methyl propanal, methyl acetate, methanol, ethyl acetate and diethyl ether. In all, there appeared to be more than 120 compounds present as shown by gas chromatography. Thirty compounds have been identified, including 9 alcohols, 7 carbonyl compounds, 6 esters, 3 terpenes, 4 aromatic compounds and benzoic acid.

#### C. Color, Texture and Other Quality Factors

Negotiations with the New Jersey Agricultural Experiment Station, New Brunswick, on the processing characteristics of Eastern pears are nearly completed. It is expected that experimental studies will begin with the 1967 crop.

The mechanical harvesting of cherries was a decisive factor in handling the 1966 crop. Mechanical harvesting reduced labor costs to growers by more than \$1 million and made possible harvesting of about 7% of the national crop which would not have otherwise been harvested.

#### D. Technology - Process and Products Development

The explosion-puffing of four types of apple pieces, 2 sizes of dice, segments and half-segments (16ths halved transversely which are the best shape for most uses), indicated that the half-segments are the most versatile and provide highest yields. The output of this product from the explosion gun is suitable for making instant applesauce. Where uniformity is important, air classification can satisfactorily separate the small percentage of partially

puffed and irregularly shaped pieces. Porous apple pieces intended for use as snacks are normally hygroscopic, but new processing techniques are solving this problem. Blueberries can be explosion-puffed at any season if they are dried to 12-15% moisture and stored at room temperature.

Laboratory scale tests show that apples can be peeled by a combination of hot isopropyl alcohol (to remove wax) followed by treatment with dilute alkali. However, apples thus processed did not produce a satisfactory applesauce because the lye did not remove the calyx which appeared as black specks in the applesauce. Since mechanical peeling is not practical for apples below a certain size, the harvesting of undersized fruit expected from mechanical harvesting provides a powerful stimulus for the development of other peeling methods for such undersized fruit.

Irradiation by ultraviolet light appears to be a useful way to reduce a low initial microbial count in cider. In the study a 1/8-inch layer of cider was pumped through a shell surrounding the ultraviolet lamp. The exposure time varied between 11 and 40 seconds and the longer exposure reduced the microbial count by 99%. A taste-panel evaluation showed no detectable change in flavor after UV radiation. This new process may permit distributors to market fresh cider without using chemical preservatives and the development will be brought to the attention of cider producers.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

- Collins, J. L. and Wiley, R. C. 1967. Penetration and distribution of calcium ions in thermal-processed apple slices. Food Sci., 32, pp. 185-188.
- Wiley, R. C., Tavakoli, M., and Moore, M. D. 1966. Determination of hexuronic acids in fruit by gas chromatography. Proc. Am. Soc. Hort.Sci., 89, pp. 34-39.

Color, Texture and Other Quality Factors

- Harrington, W. O., Robinson, J. F., Hills, C. H., and Hewetson, F. N. 1966. Effect of cultural practices on processed cherry quality. Proc. Am. Soc. Hort. Sci., 88, pp. 184-189.

Technology -- Process and Product Development

- Dougherty, R. H., Modery, M. G., and Wiley, R. C. 1966. Effect of vacuum time and steam blanch temperature on the quality of canned apple slices. Proc. Am. Soc. Hort. Sci., 89, pp. 46-52.
- Eisenhardt, N. H., Eskew, R. K., Cording, J., Jr., Talley, F. B., and Huhtanen, C. N. 1967. Dehydrated explosion puffed blueberries. U. S. Agricultural Research Service, ARS-73-54.
- Gaston, H. P., and Levin, J. H., and Whittenberger, R. T. 1967. Machine harvesting sweet cherries. A progress report of studies being conducted in Michigan. The Great Lakes Fruit Growers News, 6, no. 5, pp. 12-14.
- Gaston, H. P., Levin, J. H., and Whittenberger, R. T. 1966. How to use cherry harvesting machines. Mich. State U., Coop. Ext. Service. Exten. Bulletin 532, 2 pp.
- Harrington, W. O., and Hills, C. H. 1966. Wax solvents improve the lye-peeling of apples. Natl. Apple Ind. Utiliz. Conf., Proceedings Report, pp. 102-103.
- Harrington, W. O., and Hills, C. H. 1966. Preservative effect of diethyl pyrocarbonate and its combination with potassium sorbate on apple cider. Food Technol., 20, pp. 1360-1362.

General

- Ogg, C. L. 1966. Determination of molecular weight. J. Assoc. Offic. Anal. Chemists, 49, pp. 744-749.
- Wiley, R. C. 1966. Apple pectin and health. National Apple Ind. Utiliz. Conference, Proceedings Report, pp. 58-60.



## AREA NO. 8. TOBACCO UTILIZATION - INDUSTRIAL PRODUCTS

Problem. Although neither food nor fiber, tobacco nevertheless is grown on about a million acres, and in seven states provided more farm cash receipts than any other field crop in 1964. The farm value is about \$1.3 billion. This crop is unique in that it yields about \$3.1 billion in Federal and State taxes. Of the problems affecting the tobacco industry, the much publicized charges concerning the effect of tobacco usage on health are the most serious. Although much controversy still surrounds these charges, the importance of the tobacco economy and the seriousness of the charges dictate that research in this area be intensified. Such a program will serve to elucidate more completely the extent of smoking-health relationships and the capabilities of research to alter the observed physiological effects of smoke on animal tissue. Information obtained in such studies may also be of value in other industrial problems, such as the determination of relationships between the chemical composition of tobacco and smoke, and the overall quality of tobacco products. It should be noted that the present program represents a significant reorientation of effort from past endeavor concerned mainly with quality problems.

### USDA AND COOPERATIVE PROGRAM

The Department has an expanding program involving many facets of the chemistry and biology of tobacco and its smoke. Much of the work is basic in nature and, although the program is health-oriented, many findings of value in industrial problems not related to health may be forthcoming. The present program is divided into six general areas: basic studies on the composition of cigarette smoke; similar investigations on tobacco leaf; biochemical changes during fermentation and aging; the nature of the pyrolytic products from leaf substances or fractions; the effect of chemical additives on the composition of cigarette smoke; and biomedical studies related to the biological assaying of cigarette smoke.

The Federal scientific effort devoted to research in this area totals 36.8 scientist man-years, including 21 of contract research. This effort is applied as follows:

Chemical composition, physical properties and structure investigations involves 5.4 scientist man-years at Wyndmoor, Pennsylvania, on composition of cigarette smoke and acids and bases in cigar smoke.

Contract research includes 1.4 scientist man-years effort at Durham, North Carolina, on a study of neutral resins of tobacco leaf and three projects at the University of Kentucky, Lexington, totaling 3.2 scientist man-years on aromatic hydrocarbons, heterocyclic bases and nitrosamines in smoke.

Chemical and physical investigations to improve products involves a total of 15.6 scientist man-years, including 11.6 of contract research at the

University of Kentucky. The program includes evaluation of cigarette modifiers, study of pyrolytic products and development of assaying procedures with emphasis on improved biological assaying methods.

Microbiology and fermentation involves 5.2 scientist man-years at Wyndmoor on a study of biochemical changes in tobacco during aging and fermentation.

Research on technology - process and product development involves a total of 6.0 scientist man-years. This effort includes 1.2 scientist man-years at Wyndmoor on large-scale production of samples for biological assays and other studies, 1.2 scientist man-years of contract research with Houdry Process and Chemical Company, Linwood, Pa., to develop additives to modify cigarette burn temperatures, contract research of 0.9 scientist man-year at Health Research Institute, Buffalo, N. Y., and 1.3 at University of Kentucky pertaining to application of bioassay methods, and 1.4 at the University of Kentucky for production of experimental cigarettes and preparation of smoke condensates therefrom.

#### PROGRAM OF STATE EXPERIMENT STATIONS

A total of 8.9 scientist man-years is devoted to this area of research.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Chemical Composition, Physical Properties and Structure

1. Composition of tobacco smoke. Significant chemical differences were found in the smoke of four custom cigars which varied only in the origin (Pennsylvania, Puerto Rico, Columbia and Dominican Republic) of the unblended filler tobacco. Quantitative comparisons were based on analysis of more than 30 compounds. Smoke from cigars made with Pennsylvania filler contained the highest levels of nicotine and related alkaloids. Smoke from the Columbian filler was highest in isoprene and related terpenes and in phenylacetic acid. Dominican Republic tobacco smoke was lower in carboxylic acids than the other three. The Columbian cigar is the most chemically unique and appears, based on preliminary subjective panel testing, to be the most characteristic in smoking quality.

In research on the composition of cigarette smoke, work has continued on the study of structure and properties of the high molecular weight pigment isolated from smoke condensate. A subfraction of the pigment was demonstrated to contain a silicone and 22 heterocyclic bases, including nicotine and four other alkaloids.

This represents the first report of the occurrence of nicotine and other alkaloids found in high molecular weight form in cigarette smoke.

In other studies of the composition of smoke condensate, four aromatic amines were identified: N-phenyl-2-naphthylamine, N-phenyl-4-isopropylphenylamine, diphenylamine and 9,9-dimethylacridan.

Except for aniline, the occurrence of aromatic amines in cigarette smoke condensate has not previously been shown.

In contract research at the University of Kentucky Research Foundation, Lexington, selective fractionation and separation of the polynuclear aromatic hydrocarbons in cigarette smoke condensate showed the presence of more than 20 compounds. Among those identified are phenanthrene, fluoranthene, pyrene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, and dibenzo(a,i)pyrene. Benzo(a)pyrene and other polymeric aromatic hydrocarbons were detected in quantities as low as  $10^{-8}$  gram.

The literature values for benzo(a)pyrene in cigarette smoke are, however, probably lower than the content of this compound in the smoke. In developing an improved method for the determination of polynuclear hydrocarbons in cigarette smoke, the separation of a mixture of known amounts of authentic compounds was investigated. Data obtained in the recovery studies showed that the procedures had certain limitations and that losses were incurred during chromatographic separations.

2. Composition of tobacco leaf. In contract research at the Research Triangle Institute, at Durham, North Carolina, on the "neutral resins" of tobacco leaf, levulinic acid was isolated from this source for the first time. Several other acids are indicated to be constituents of tobacco leaf. In another phase of the research to separate components of the complicated neutral resin fraction, a countercurrent distribution procedure was applied to a subfraction to obtain esters and non-polar alcohols, alkaloids and polar alcohols.

Cigar leaf tobaccos grown in different geographical locations were found to have characteristic differences in chemical composition. Pennsylvania filler was lowest in solanesol and the Columbian filler was highest in unidentified constituents. The free amino acid pattern in the Pennsylvania and Columbian filler was also distinctly different from the Puerto Rican and Dominican types.

## B. Chemical and Physical Investigations to Improve Products

1. Cigarette modifiers. Cooperative research at the University of Kentucky, Lexington, on the evaluation of cigarette modifiers suggests that the amount of at least one deleterious substance in cigarette smoke (benzo(a)pyrene) can be altered by crop fertilization. Cigarettes manufactured from burley tobacco grown under low and high levels of nitrate fertilization were analyzed and the latter were found to have a 17-fold greater content of naturally occurring nitrate. The amount of benzo(a)pyrene in the smoke of high nitrate fertilized tobacco was only  $0.85 \mu\text{g}/100$  cigarettes. For the low nitrate fertilized tobacco, however, the benzo(a)pyrene content of the smoke was  $6.1 \mu\text{g}/100$  cigarettes. It therefore appears that less benzo(a)pyrene is produced when tobacco high in nitrate is burned.

2. Pyrolytic products. Pyrolysis studies with components of tobacco leaf provide a basis for correlating leaf source with product constituents in smoke. Tobacco leaf pigment has been shown, for the first time, to be a highly efficient phenol precursor. Other pyrolytic sources of phenol include lignin, pectin, cellulose, lysine and phenylalanine. The latter sources are part of the results from research on pyrolysis of amino acids at the University of Kentucky Research Foundation. At temperatures simulating that of a burning cigarette, benzo(a)pyrene and other polynuclear aromatic compounds are produced. Compounds studied included lysine monohydrochloride, leucine, phenylalanine, tryptophan and pyrrole. Low molecular weight carbon compounds, such as methane, ethylene, acetylene, and carbon monoxide, were identified in the gas evolved during pyrolysis of tryptophan and lysine.

3. Biological assay methods. In contract research at the University of Kentucky Research Foundation, Lexington, progress was made in the various independent biomedical studies which may serve as a basis for the development of new and improved biological assay methods. Currently, rat tracheal epithelial rings can be cultured for periods up to 10 days. This has permitted a day-to-day study of characteristic cell changes over this period.

Results of initial studies on the effect of smoking on saliva showed that the thiocyanate ion (SCN) concentration was significantly higher in saliva from smokers. The antibacterial activity of smokers' saliva to Lactobacillus acidophilus has been correlated with the thiocyanate concentration. Techniques developed in this study will be useful in determining the antibacterial effect of smokers' saliva on other organisms of the oral cavity.

A technique for evaluating absorption through the cheek pouch of hamsters has been developed.

Research to study the apparent tumor-inducing property of viruses has been continued. In addition to the previously reported activity of influenza virus, other studies indicate that a virus (or virus-like agent) is associated with methylcholanthrene-induced fibrosarcomas in mice.

The oral administration of phenanthrene, a possible anticarcinogen, prior to the oral administration of labelled benzo(a)pyrene results in decreased amounts of benzo(a)pyrene in certain target tissues. The basis for this effect of phenanthrene is unknown, but presumably involves competition for transport mechanisms or binding sites in the target tissue.

In research on absorption of carcinogens and structurally-related noncarcinogens, investigations were conducted with 3-methylcholanthrene to develop procedures for detecting polycyclic hydrocarbons in the lungs, stomach, and small and large intestines of rodents.

Studies have been initiated to compare the suitability of the colchicine and tritiated thymidine techniques for determining respiratory cell turn-over rates in inbred mice. With each agent the first evidence of mitotic activity



was found six hours after the agent was injected. Tissue sections were examined to establish how much lung tissue has to be studied and whether one area differs from another. Bronchial epithelial cells from primary, secondary and tertiary bronchi were counted and the minimum number of cells which must be counted to determine cell turnover was established. This information should be of value in expediting valid determinations of rates.

#### C. Microbiology and Fermentation

A project to study the biochemistry of tobacco fermentation and aging was initiated recently. Aqueous extracts of fermented tobacco leaf appear to be extremely low in protein content and high in carbohydrate.

An investigation was begun on the possible presence of mycotoxins in tobacco. Thin-layer chromatography of extracts of mold-infected tobacco leaf indicate the presence of components apparently not found in normal tobacco.

#### D. Technology - Process and Product Development

1. Modification of cigarette burn temperature. The study of the effect of additives on the burn temperature of cigarettes was continued under a contract at Houdry Process and Chemical Company, Marcus Hook, Pennsylvania. The addition of elemental sulfur or benzothiazyl disulfide to cigarette tobacco caused an elevation of burn temperature.

The most successful depressions of cigarette burn temperatures to date have been obtained with lead borate glass (previously reported), basic magnesium carbonate and nickel oxalate. A large number of cigarettes containing these burn temperature depressants were prepared for studies on changes in smoke condensate composition at the University of Kentucky.

2. Production of experimental cigarettes and smoke condensates. Contract research at the University of Kentucky Research Foundation, Lexington, provides for manufacturing experimental cigarettes and for preparing cigarette smoke condensates. The cigarette-making machines have not arrived, but two of the smoking machines have been received and are in operation.

Cigarettes manufactured by a commercial company from burley tobacco grown under high and low rates of nitrogen fertilization were received from the Crops Research Division. These cigarettes were smoked and condensates prepared for use in composition and bioassay studies. (See results reported under B-1, cigarette modifiers.)

Large-scale fractionations of smoke condensate, currently totaling about 6 kilos, have been conducted at Wyndmoor to supply samples for the bioassay test by Health Research, Inc., at Orchard Park (Buffalo), New York.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition, Physical Properties and Structure

- Chamberlain, W. J., and Stedman, R. L. 1966. Composition studies on tobacco. XXIV. High molecular weight pigment in smoke of various tobaccos. Tobacco Sci., 10, pp. 162-163.
- Dymicky, M., Chortyk, O. T., and Stedman, R. L. 1967. Composition studies on tobacco. XXVII. Polyphenol-amino acid leaf pigment: further structural investigations. Tobacco Sci., 11, pp. 42-44.
- Miller, R. L., Lakritz, L., Dooley, C. J., and Stedman, R. L. 1967. Composition studies on tobacco. XXVI. Aryl amines in the nitromethane-soluble neutral fraction of smoke condensate. Tobacco Sci., 11, pp. 35-36.
- Osman, S. F. and Barson, J. L. 1966. Hydrocinnamyl nitrile in cigar smoke condensate. Chem. & Ind. p. 699.
- Osman, S. F., and Barson, J. L. 1966. An aromatic acid fraction of cigar smoke condensate. Phytochemistry 5, pp. 511-515.
- Osman, S. F., and Barson, J. L. 1966. The isolation of farnesyl acetone from cigar smoke condensate. Tobacco Sci., 10, pp. 85-87.
- Osman, S. F., and Barson, J. L. 1967. Solvent fractionation of Girard T derivatives of carbonyl compounds using dimethyl sulfoxide. Anal. Chem. 39, pp. 530-531.
- Schmeltz, I., Dooley, C. J., Stedman, R. L., and Chamberlain, W. J. 1967. Composition studies on tobacco. XXII. The nitromethane-soluble, neutral fraction of cigarette smoke. Phytochemistry 6, pp. 33-38.
- Stedman, R. L., Chamberlain, W. J. and Miller, R. L. 1966. High molecular weight pigment in cigarette smoke. Chem. & Ind., pp. 1560-1562.
- Stedman, R. L., and Miller, R. L. 1967. Alkylating activity of cigarette smoke condensate. Chem. & Ind., pp. 618-619.

Chemical and Physical Investigations to Improve Products

- Bortner, C. E., and Ogg, C. L. 1966. Determination of oven moisture in tobacco. J. Assoc. Offic. Anal. Chemists, 49, pp. 525-528.

- Chortyk, O. T., Schlotzhauer, W. S., and Stedman, R. L. 1966. Composition studies on tobacco. XXIII. Pyrolytic and structural investigations on the polyphenol-amino acid pigments of leaf. Beitr. Tabakforsch., 3, pp. 422-429.
- Schlotzhauer, W. S., Schmeltz, I., and Hickey, L. C., 1967. Pyrolytic formation of phenols from some high molecular weight tobacco leaf constituents and related non-tobacco materials. Tobacco Sci., 11, pp. 31-34.
- Schlotzhauer, W. S., and Schmeltz, I. 1967. Non-alkaloidal bases from pyrolysis of tobacco leaf pigment at the approximate burn temperature of a cigarette. Tobacco Sci., 11, pp. 89-90.
- Swain, A. P., Peterson, R. F., Jr., and Stedman, R. L. 1966. Composition studies on tobacco. XXI. The headspace vapors of leaf. J. Sci. Food Agr. 17, pp. 349-353.

## AREA NO. 9. MAPLE SAP AND SIRUP UTILIZATION - FOOD

Problem. The extensive unused stands of sugar maple trees are largely located in agriculturally depressed areas that are commonly devoted to small-scale dairy farming. Since only a small percent of the available sugar maple trees are presently tapped for sap production, and about 50% of the sirup consumed in the United States is imported, untapped sugar maples represent a good potential source of increased cash income for farmers in these areas. The maple area includes 14 states from Minnesota to Maine and south to Virginia. Under proper conditions, maple sirup can be a six-weeks seasonal crop not in competition with other farm activities and with a per acre value equal to or exceeding that of other farm products. Based largely on recent research carried out in the Department and the State Experiment Stations, the methods of collecting and processing sap into sirup are being streamlined. This has resulted in greatly increased efficiency and larger hourly returns to the sirup producer for his labor. The advent of tube collection and transportation of sap has reduced the cost of sap handling 40% and has eliminated much hand labor.

Oil-firing of evaporators and improved systems of steam removal have provided efficient and sanitary plants. The taphole germicidal pellets and sanitary methods of sap handling have tended to stabilize crop yields and standardize sirup quality. While the results of previous research have contributed to modernization of the industry, much more information is needed so that all operations for the production of high-quality maple sirup and other maple products can be conducted in a predictable, efficient manner. Not only can the low income farms be greatly benefited, but the existing maple industry can be put on a higher economic plane and modernized to be made competitive with other crop and livestock farming to bring about improved land use.

### USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists and microbiologists. These scientists are engaged in both basic and applied research in investigations concerned with the problems of improving sap handling and processing, producing high-quality maple sirup, and developing new outlets for all maple products while lowering the cost of the product. Most of this work is conducted at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 4.0 scientist man-years. Of this number, research on chemical composition and physical properties comprises 1.0, research on microbiology and toxicology comprises 1.0, and research on technology--process and product development comprises 2.0, including 0.1 in contract research on sap storage with J. L. Sipple & Son, Bainbridge, New York. In the research work, cooperation is maintained with personnel of the Federal Extension Service in maple-producing states and with Cornell University.



## PROGRAM OF STATE EXPERIMENT STATIONS

A total of 1.5 scientist man-years is devoted to this area of research.

### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

#### A. Chemical Composition and Physical Properties

Methyl ethyl ketone can extract flavor precursors from maple sirup that has been extracted with chloroform, which removed maple flavor. The concentrated methyl ethyl ketone extract has a sweet, slightly maple-like odor. However, an attempted GLC separation revealed no compounds contributing to the flavor. This suggests that (a) the material in the extract is very low in volatility or (b) is in such small amounts as to escape detection. When the flavor-containing chloroform extract of maple sirup is subjected to a GLC-mass spectrograph tandem procedure the more volatile part of the extract was found to have small amounts of many compounds. Further work must be done to determine whether these are artifacts, contaminants or real constituents of maple flavor.

#### B. Microbiology and Toxicology

Ultraviolet irradiation is an effective method for controlling microbial growth in maple sap. A test microbial population of  $2 \times 10^6$  organisms per ml., was reduced to 0.9% of this count by ultraviolet radiation and a recirculation rate sufficient to effect complete recirculation of the sap in a 60-gallon test vessel in two hours. Hypochlorite sanitizers providing 50 parts per million of chlorine were found to be effective in sanitizing reverse osmosis apparatus. Fifteen minutes exposure reduced microbial counts over  $10^6$  organisms per ml. to less than 10 organisms per ml. However, the high pH of the hypochlorite sanitizers have a deleterious effect on the reverse osmosis membranes. This effect can be minimized by washing the membranes with acetate buffer at pH 4.5.

#### C. Technology - Process and Product Development

1. Maple sap storage. Maple sap irradiated by inline ultraviolet lamps as it was run into storage, followed by continuous irradiation of the surface of the stored sap showed no deterioration after one week of storage. The grade of the sirup produced was identical with sirup made from fresh sap. Sap treated by surface irradiation only had a higher microbial count and produced sirup at least one grade darker than fresh sap. The control sample, sap stored without exposure to ultraviolet radiation, had a high yeast and bacterial count at the end of one week and the sirup produced was at least two grades darker than sirup made from fresh sap.

2. Reverse osmosis. A four-module reverse osmosis unit was tested on fresh sap of the 1966 season. The data confirmed exploratory experiments. Sap was successfully concentrated from its original Brix of  $2.5^{\circ}$  to  $10^{\circ}$ , that is, 75% of the water was removed by reverse osmosis. The  $10^{\circ}$  Brix concentrate when concentrated to  $65.5^{\circ}$  Brix sirup by conventional boiling had full-bodied

maple flavor with no detectable foreign flavor. The loss of sap solids in the byproduct water was approximately one part in 2000.

A pilot plant reverse osmosis unit under construction is designed to handle up to 10,000 gallons of maple sap per 24-hour day to produce a 10° - 12° Brix sap for finishing by conventional atmospheric boiling. The concentration of maple sap by thermal distillation is one of the more costly of the different steps involved in maple production. The use of reverse osmosis to remove most of the water would be a much less expensive method.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Stinson, E. E., Dooley, C. J., Purcell, J. M., and Ard, J. S. 1967.  
Quebrachitol--a new component of maple sap and sirup. J. Agr. & Food  
Chem., 15, pp. 394-397.

Microbiology and Toxicology

Kissinger, J. C., and Willits, C. O. 1966. The control of bacterial contami-  
nation in maple sap stored in field storage tanks by ultraviolet  
irradiation. J. Milk & Food Technol., 29, pp. 279-282.

Technology -- Process and Product Development

Willits, C. O., Underwood, J. C., and Merten, U. 1967. Concentration of re-  
verse osmosis of maple sap. Food Technol., 21, pp. 24-26.

PUBLICATIONS AND PATENTS - DISCONTINUED REPORTING AREA

Schepartz, A. I. 1966. Honey catalase: occurrence and some kinetic  
properties. J. Apic. Res., 5, pp. 167-176.

Scott, W. E., and Krewson, C. F. 1966. Euphorbia lagascae spreng. enzyme  
activity in the seed. J. Am. Oil Chemists Soc., 43, pp. 466-468.

White, J. W., Jr. 1966. Improving the color of beeswax. Gleaning Bee  
Culture, 94, pp. 742-743, 758.

White, J. W., Jr. March 14, 1967. Decolorizing of beeswax. U. S. Patent  
3,309,389.

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E6 2	Milk Products Utilization Investigations.			
E6 2-85 (Rev.)*	Interactions of milk proteins in solution	Wyndmoor, Pa.	Yes	1-A
E6 2-94 (C)*	Effects of nonfat dry milk on bread yeast fermentation	Madison, Wis.	Yes	1-D-2
E6 2-95(Gr)	Increased protein stability of evaporated milk: study of calcium phosphate-casein micelles	Columbus, Ohio	Yes	1-A
E6 2-96	Improving the flavor stability of anhydrous milk fat	Washington, D.C.	Yes	1-A, 1-B
E6 2-98*	Studies on stale flavor in sterile milk and development of means to prevent its formation	Washington, D.C.	Yes	1-B
E6 2-99*	Improved sterile whole milk concentrates: the production of reversible sol-gel transformations in high solids sterile concentrates	Washington, D.C.	Yes	1-A
E6 2-100(C)*	Removal of radioactive strontium from milk on a commercial scale	Springfield, Mo.	Yes	1-E-3
E6 2-101	Ribosomal nucleic acids	Wyndmoor, Pa.	Yes	1-A
E6 2-102(C)	Heat stability of individual milks	St. Paul, Minn.	Yes	1-A
E6 2-103	Enzyme studies relating to milk	Wyndmoor, Pa.	Yes	1-A
E6 2-105(Gr)	Physical changes in milk and milk concentrates associated with steam injection and bubble collapse	Raleigh, N.C.	Yes	1-E-4
E6 2-107(C)	Relation of milk fat composition, particularly fat and protein, to dietary of cow	College Park, Md.	Yes	1-A
E6 2-108(Gr)	Flavors and their precursors in milk derived from pasture or dry feeding practices	College Park, Md.	Yes	1-B
E6 2-109(Gr)	Study on the desirable flavors of butter: isolation and identification of specific flavor contributing compounds and their precursors	Corvallis, Oregon	Yes	1-B
E6 2-111(Gr)	Lactone, methyl ketones and their precursors in milk products: effects on off-flavors and development of procedures for their control	University Park, Pa.	Yes	1-B
E6 2-113*	Improvement of dried whole milk	Washington, D.C.	Yes	1-E-1
E6 2-115	New and improved processing equipment	Washington, D.C.	Yes	1-E-4
E6 2-116(C)	Determination of flavor and quality stability of commercial fluid milk during storage to permit Iodine-131 decay	Greenville, Ill.	Yes	1-E-3
E6 2-117(C)	Determination of the commercial feasibility and practicality of a combined anion-cation fixed resin bed system for removing radionuclides from milk	Springfield, Mo.	Yes	1-E-3
E6 2-118	Mechanisms of the development and maintenance of heat resistance and dormancy in bacterial spores	Washington, D.C.	Yes	1-D-1
E6 2-119	Effects of enzymes on the chemistry, antigenicity and allergenicity of milk proteins	Washington, D.C.	Yes	1-C
E6 2-120	Identification of the principal proteins in cow's milk responsible for allergy to milk	Washington, D.C.	Yes	1-C
E6 2-121**	Composition and primary structure of purified milk proteins	Wyndmoor, Pa.	Yes	1-A



LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E6 2-122**	Chemical and enzymatic modification of milk proteins	Wyndmoor, Pa.	Yes	1-A
E6 2-123**	Properties of the protein complexes in milk	Wyndmoor, Pa.	Yes	1-A
E6 2-124**	Interactions of $\alpha_s$ - and $\kappa$ -casein	Wyndmoor, Pa.	Yes	1-A
E6 2-125**	Relationship of milk, colostrum and blood proteins	Washington, D.C.	No	
E6 2-126	Assay methods for milk allergens	Washington, D.C.	Yes	1-C
E6 2-127**	Improving the physical stability of sterilized milk	Washington, D.C.	Yes	1-C
E6 2-128**	Fundamental study of factors influencing the distribution of antioxidant in fat-containing dairy products	Washington, D.C.	Yes	1-E-1
E6 2-129**	Water vapor absorption by dry milk products	Washington, D.C.	Yes	1-E-1
E6 2-130**	Methods for the isolation and characteristics of flavor constituents and their precursors in natural products	Washington, D.C.	Yes	1-A
E6 2-131**	Influence of air pollution on flavor of dry dairy products	Washington, D.C.	Yes	1-B
E6 2-132 (Gr)**	Heat transfer in powdered milk	East Lansing, Mich.	No	
E6 2-133**	Pilot plant studies on natural and processed skim milk cheeses	Washington, D.C.	Yes	1-E-2
E6 2-134**	Survival of Salmonella in cheese and other dairy products	Washington, D.C.	No	
E6 2-135**	Chemical changes occurring in sterile milks resulting in the development of off-flavors during storage	Washington, D.C.	No	
E6 2-136**	Improving the flavor stability of sterilized milks	Washington, D.C.	No	
UR-A7-(60)-5	Milk coagulating enzymes	Punjab, India	Yes	1-E-2
UR-A7-(60)-11	Sulfur compounds in relation to flavor and stability of milk	Punjab, India	Yes	1-B
UR-A7-(60)-13*	Buffalo milk in cheese manufacturing	Anand, India	Yes	1-E-2
UR-A7-(60)-16	Phosphoproteins of milk	Bangalore, India	Yes	1-A
UR-A7-(60)-22	Protease-peptone fraction of milk	Punjab, India	Yes	1-A
UR-A7-(60)-48	Dipicolinic acid synthesis in bacterial spores	Pantnagar, District Mainital, India	Yes	1-D-1
UR-A7-(60)-105	Starter bacteria and variants in development of cheese flavor	Punjab, India	Yes	1-D-1, 1-E-2
UR-A10-(60)-37	Formation of unnatural nucleic acids	Haifa, Israel	Yes	1-A
UR-A10-(60)-61**	Immunological reactions of infants to cow's milk	Jerusalem, Israel	No	
UR-E3-(60)-7	Structures and interactions of nucleic acid	Graz, Austria	Yes	1-A
UR-E8-(60)-16	Dietary factors controlling flavor in milk	Helsinki, Finland	Yes	1-B
UR-E9-(60)-46*	Nonprotein nitrogenous constituents of milk	Paris, France	Yes	1-A

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
UR-E9-(10, 60)-80*	Sub-unit structure of nucleic acids	Strasbourg, France	Yes	1-A
UR-E10-(60)-3	Surface changes in fat globules of dried whole milk	Berlin, W. Germany	Yes	1-E-1
UR-E21-(60)-21	Mechanisms of cheese-ripening process	Olsztyn, Poland	Yes	1-A
UR-E25-(60)-37	Thermal properties of milk	Madrid, Spain	No	
UR-E26-(60)-9	Methods for purification of protein complexes	Uppsala, Sweden	Yes	1-A
UR-S3-(60)-10*	Structure and properties of proteolytic enzymes	Rio de Janeiro, Brazil	Yes	1-A

\* Discontinued during reporting year.

\*\* Initiated during reporting year.

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E6 5	Meat Utilization Investigations.			
E6 5-17 (Rev.)	Chemical, physical, and biological factors involved in the development of rancidity in fats, fatty tissues, and meats	Beltsville, Md.	Yes	2-C-1
E6 5-19 (Rev.)*	Studies on the recovery and identification of substances responsible for flavor and aroma in meat	Wyndmoor, Pa.	Yes	2-B
E6 5-20 (Rev.)*	Chemical reactions involved in meat-curing	Wyndmoor, Pa.	Yes	2-C-2
E6 5-21(C)	A histochemical study of components of meat connective tissues and their relation to tenderness	Baton Rouge, La.	Yes	2-A
E6 5-22(Rev.)	Studies of psychrophilic microorganisms	Beltsville, Md.	Yes	2-D-1
E6 5-23	Improving the flavor of cured meats through a study of the interrelationships of temperature, curing substances, microbial metabolism and mutation rates	Beltsville, Md.	Yes	2-D-2
E6 5-24 (Rev.)	Meat protein composition and distribution in relation to tenderness and juiciness	Wyndmoor, Pa.	Yes	2-A
E6 5-26(C)	Fungi associated with meat processing and flavor development	Ames, Iowa	Yes	2-D-2
E6 5-27	Identifying substances in wood smoke that contribute to the flavor and aroma of meats	Wyndmoor, Pa.	Yes	2-B
E6 5-28	Development of new or improved meat processing methods and of new meat products	Wyndmoor, Pa.	Yes	2-E-1
E6 5-30(C)	Development of new smoked meat products	East Lansing, Mich.	Yes	2-E-2
E6 5-31(C)	New frozen meat products and their time-temperature relationships	Columbia, Mo.	Yes	2-E-2
E6 5-32(Gr)	The relationship of amounts and ratios of heme pigments to oxidative rancidity	Tallahassee, Fla.	Yes	2-C-2
E6 5-33	The development of an accurate laboratory method of estimating the thermal history of meat products	Wyndmoor, Pa.	Yes	2-E-1
E6 5-34(Gr)	A study of the nature and significance of noncarbonyl volatile compounds associated with rancidity in meats	New Brunswick, N.J.	Yes	2-C-1
E6 5-35(C)	Development of new ready-to-eat meat products suitable for production in small, rural industries	Baton Rouge, La.	Yes	2-E-2
E6 5-36(C)	Reactions of muscle proteins as they relate to the thermal effects of meat processing and large-scale institutional cookery	Ithaca, N. Y.	Yes	2-E-2
E6 5-37(C)**	New lamb and mutton products	University Park, Pa.	No	
E6 5-38(Gr)**	Effect of indigenous microflora on growth and toxin production of <u>Staphylococcus aureus</u> and food poisoning <u>Clostridia</u> in semi-preserved meats	Davis, California	No	
UR-A6-(60)-4	New semi-dehydrated fried meat products	Taichung, Taiwan	Yes	2-E-2
UR-E8-(60)-14	Influence of fats on flavor and aroma of dry sausage	Helsinki, Finland	Yes	2-E-1
UR-E19-(60)-17	The use of protozoa to detect harmful substances in meat	Utrecht, Netherlands	Yes	2-D-2
UR-E21 (60)24	Antioxidant components of wood smoke used in meat-curing	Gdansk, Poland	Yes	2-B
UR-E29-(60)-70	Specific reducing systems in pork muscle	Surrey, England	Yes	2-C-2

\* Discontinued during reporting year.

\*\* Initiated during reporting year.

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E6 3	Animal Fats and Oils and Special Products. Utilization Investigations.			
E6 3-55 (Rev.)*	Long-chain fat derivatives for polymer modification	Wyndmoor, Pa.	Yes	3-B-1
E6 3-58 (Rev.)*	Soap-detergent combinations based on animal fats	Wyndmoor, Pa.	Yes	3-B-2
E6 3-65*	Fractionation and analysis of lipids	Wyndmoor, Pa.	Yes	3-A
E6 3-66*	Polymerizable amides from animal fats	Wyndmoor, Pa.	Yes	3-B-1
E6 3-69 (C)(Rev.)	Spatial interrelations within triglyceride molecules	Villanova, Pa.	Yes	3-A
E6 3-70 (C)	X-ray investigations of a mixed triglyceride	Villanova, Pa.	Yes	3-A
E6 3-71 (C)	Interfacial adsorption characteristics of salts of alkyl esters of $\alpha$ -sulfo fatty acids as related to their wetting and detergent action	Bethlehem, Pa.	Yes	3-B-2
E6 3-72	Development of industrially useful chemicals by free radical addition products	Wyndmoor, Pa.	Yes	3-B-3
E6 3-73	Biodegradable detergents from animal fats	Wyndmoor, Pa.	Yes	3-B-2
E6 3-74 (Gr)	Synthesis of pure glycerides	Storrs, Conn.	Yes	3-A
E6 3-75 (Gr)	Ozonization of animal fats	Austin, Minn.	Yes	3-B-4
E6 3-76*	New mathematical approaches to physical measurement	Wyndmoor, Pa.	Yes	3-A
E6 3-77 (C)	Additive chlorination and hydrogenolysis of animal fats	Minneapolis, Minn.	Yes	3-B-2
E6 3-78 (C)	High pressure hydrolysis of animal fats to alcohols without simultaneous chain saturation	Chicago, Ill.	Yes	3-B-2
E6 3-80	Synthesis of chemical intermediates by the introduction of reactive ester, sulfur, and oxygen functional groups into animal fats	Wyndmoor, Pa.	Yes	3-B-4
E6 3-81 (Gr)	Biological synthesis of unsaturated fatty acids	Not yet determined	No	
E6 3-82	Structure of animal fat constituents and derivatives by physical methods	Wyndmoor, Pa.	Yes	3-A
E6 3-83	Development from animal fats of synthetic lubricants by the preparation of small ring heterocyclic derivatives	Wyndmoor, Pa.	Yes	3-B-3
E6 3-84**	Incorporation in polymers of fat derivatives bearing functions capable of cross-linking, plasticizing or stabilizing	Wyndmoor, Pa.	Yes	3-B-1
E6 3-85**	Physicochemical separation of animal lipids	Wyndmoor, Pa.	Yes	3-A
E6 3-87**	Relation of structure of fatty monomers to glass and melting transitions of their polymers and copolymers with commercial monomers	Wyndmoor, Pa.	No	
UR-E-21-(40,60)-28	Kinetics and thermodynamics of fat autoxidation	Gdansk, Poland	Yes	3-B-4
UR-E-25 (60)-22	Cocoa butter substitutes from animal fats	Madrid, Spain	No	
UR-A-7 (60)-72	Preparation and properties of long chain sulfated monoglycerides	Bombay, India	Yes	3-B-2
UR-E-9 (60)-79	Hydroxylated fatty derivatives	Marseille, France	Yes	3-B-4



LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
UR-E9- (60)-88	Autoxidation of fat at low temperatures	Paris, France	Yes	3-B-4
UR-E9- (60)-89	Polyhalogenated fatty acids and their derivatives	Paris, France	Yes	3-B-2
UR-E21- (60)-29	Thermally stable stationary phases for gas-liquid chromatography	Gdansk, Poland	Yes	3-A

\* Discontinued during reporting year.

\*\* Initiated during reporting year.

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E6 4	Hides, Skins and Leather Utilization Investigations.			
E6 4-36*	Effect of electrolytes and lipid components on hide properties	Wyndmoor, Pa.	Yes	4-A-1
E6 4-37(C)*	Noncollagenous proteins of cattlehides	Cincinnati, Ohio	Yes	4-B-3
E6 4-38 (C)(Rev)	Preparation and properties of dispersed collagen sols	Kansas City, Mo.	Yes	4-A-1
E6 4-39*	Microscopic investigation of skin and leather structure	Wyndmoor, Pa.	Yes	4-A-2
E6 4-40(C)*	Abnormalities of leather characterized by a depleted mushy texture	Cincinnati, Ohio	Yes	4-A-2
E6 4-41*	Physical properties of collagen and leathers	Wyndmoor, Pa.	Yes	4-A-2
E6 4-42*	Addition of new reactive sites to hide proteins	Wyndmoor, Pa.	Yes	4-B-1
E6 4-43	Chemical modification of hides with aldehydes in combination with phenols, amides, hydrazides	Wyndmoor, Pa.	Yes	4-B-2
E6 4-44	Chemical modification of animal hides with cyclic urea derivatives such as urons and triazones	Wyndmoor, Pa.	Yes	4-B-2
E6 4-45(Gr)	Physical properties of collagen	Chicago, Ill.	Yes	4-A-1
E6 4-48***	Regenerated collagen products for use in food products	Wyndmoor, Pa.	Yes	4-C-4
E6 4-49	Dehydration of animal hides and skins	Wyndmoor, Pa.	Yes	4-B-3
E6 4-50	New tanning processes from application of new chemical modification agents	Wyndmoor, Pa.	Yes	4-C-1
E6 4-51**	Structure of animal proteins	Wyndmoor, Pa.	Yes	4-A-1
E6 4-52 (C)**	Collagen subunits and polymers	Chicago, Ill.	No	-
E6 4-53**	Development of food uses for collagen to create new markets for hides	Wyndmoor, Pa.	Yes	4-C-4
E6 4-54**	Solubilization and reconstitution of collagen	Wyndmoor, Pa.	No	-
UR-A7-(60)-17	Polyphenolic tanning compounds	Madras, India	Yes	4-B-2
UR-A7-(60)-18	Relation of hide quality to tanning rate	Madras, India	Yes	4-C-3
UR-A7-(60)-42	Preparation and determination of the physico-chemical properties of polypeptidyl derivatives of collagen	Madras, India	No	-
UR-A7-(60)-43	Hydrothermal shrinkage of collagen and leather	Madras, India	Yes	4-A-2
UR-A7-(60)-80	Radioactive tracer study of mineral tanning	Madras, India	Yes	4-C-2
UR-A7-81	The comfort properties of shoe leathers	Madras, India	Yes	4-A-2
UR-A7-92	Rapid tannage of sole leather	Madras, India	Yes	4-C-3
UR-E8-(60)-17	Basic investigations on the structure, biosynthesis and maturation of collagen	Turku, Finland	Yes	4-A-1
UR-E19-(60)-13	Kinetics of chrome tanning	Waalwijk, Holland	Yes	4-C-2
UR-E29-(60)-67	Chemically reactive compounds for improving leather stability	Surrey, England	Yes	4-B-2

\* Discontinued during reporting year.

\*\* Initiated during reporting year.

\*\*\* Superseded by E6 4-53.

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E3 6	Potato and Other Vegetable Utilizations - Eastern Region.			
E3 6-44	Basic composition studies on the lipid fraction of potatoes	Wyndmoor, Pa.	Yes	5-A-1
E3 6-45	Basic studies on the formation and identity of the after-cooking discoloration pigment	Wyndmoor, Pa.	Yes	5-A-3
E3 6-46	Color and texture of frozen French fries	Wyndmoor, Pa.	Yes	5-B-1
E3 6-47	Effect of varietal, cultural and other source factors on the quality of processed potato products	E. Grand Forks, Minn.	Yes	5-A-4
E3 6-48	Pilot plant investigations on methods for producing dehydrated potato pieces capable of rapid rehydration by modification of internal structure and components related to texture	Wyndmoor, Pa.	Yes	5-C-1
E3 6-49	Basic studies on proteins of potatoes	Wyndmoor, Pa.	Yes	5-A-2
E3 6-50	Pigments formed during frying of chips	Wyndmoor, Pa.	Yes	5-B-2
E3 6-51	Increased utilization of mushrooms through improved processing	Wyndmoor, Pa.	Yes	6-A-2
E3 6-52	Pilot plant investigations of methods for producing dehydrated vegetable products in piece form capable of rapid rehydration	Wyndmoor, Pa.	Yes	6-A-1
E3 6-53 (C)**	Amino acid recovery from processing wastes	Not yet determined	-	-

\*\* Initiated during reporting year.

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E3 3	Apples and Other Fruit Utilization Investigations - Eastern Region			
E3 3-34*	Improvement of processed red tart cherries	Wyndmoor, Pa.	Yes	7-B, 7-C,
E3 3-35 (C)*	The relationship of apple cell wall constituents to texture of processed apple products	College Park, Md.	Yes	7-A
E3 3-36(Gr)	Radioactive tracer studies on red tart cherries	Philadelphia, Pa.	Yes	7-A
E3 3-37	Improved cider and cider products	Wyndmoor, Pa.	Yes	7-D
E3 3-38**	Improved procedures for peeling apples	Wyndmoor, Pa.	Yes	7-D
E3 3-39**	Pilot plant investigations of methods for producing dehydrated fruit products in piece form capable of rapid rehydration	Wyndmoor, Pa.	Yes	7-D
E3 3-40 (C)**	Processing characteristics of Eastern pears	New Brunswick, N.J.	Yes	7-C

\* Discontinued during reporting year.

\*\* Initiated during reporting year.



LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E5 3	Tobacco Investigations.			
E5 3-5*	Acids and bases in cigar smoke	Wyndmoor, Pa.	Yes	8-A-1
E5 3-6*	Composition of cigarette smoke	Wyndmoor, Pa.	Yes	8-A-1
E5 3-7*	Composition of oxidation products	Wyndmoor, Pa.	Yes	8-B-2
E5 3-8	Investigations of neutral resin	Durham, N. C.	Yes	8-A-2
(C)(Rev.)				
E5 3-9	Modifications of cigarette burn temperature	Linwood, Pa.	Yes	8-D-1
(C)				
E5 3-10	Evaluation of cigarette modifiers	Lexington, Ky.	Yes	8-B-1
E5 3-11(C)	Improved method for polynuclear hydrocarbons in smoke	Lexington, Ky.	Yes	8-A-1
E5 3-12(C)	Pyrolysis products of amino acids	Lexington, Ky.	Yes	8-B-2
E5 3-13(C)	Heterocyclic bases of cigarette smoke	Lexington, Ky.	No	
E5 3-14(C)	Improved biological assaying	Lexington, Ky.	Yes	8-B-3
E5 3-15(C)	Routine biological assaying	Lexington, Ky.	No	
E5 3-16(C)	Biological assaying of tobacco leaf, smoke and pyrolysates	Buffalo, N. Y.	No	
E5 3-17(C)	Analysis of tobacco and smoke condensates	Lexington, Ky.	No	
E5 3-18(C)	Production of experimental cigarettes and preparation of cigarette smoke condensates	Lexington, Ky.	Yes	8-C-2
E5 3-19(C)	Distribution and metabolism of benzo(a)pyrene	Lexington, Ky.	Yes	8-B-3
E5 3-20(C)	Electron transport system of <u>Bacteroides melaniogenicus</u>	Lexington, Ky.	No	
E5 3-21(C)	Absorption of carcinogens and noncarcinogens from gastro-intestinal tract of rodents	Lexington, Ky.	Yes	8-B-3
E5 3-22(C)	Respiratory epithelial cell turnover	Lexington, Ky.	Yes	8-B-3
E5 3-23(C)	Nitrosamines in cigarette smoke	Lexington, Ky.	No	
E5 3-24**	Isolation and identification of new physiologically active compounds in tobacco smoke	Wyndmoor, Pa.	Yes	8-A-1
E5 3-25**	Large-scale production of tobacco samples for the biological assaying of leaf, smoke, pyrolysates and fractions thereof	Wyndmoor, Pa.	Yes	8-D-2
E5 3-26**	Neutral constituents of cigar smoke from different cigar types	Wyndmoor, Pa.	Yes	8-A-1, 8-A-2
E5 3-27**	Pyrolysis of selected tobacco leaf constituents and related materials	Wyndmoor, Pa.	Yes	8-B-2
E5 3-28**	Biochemical changes in tobacco brought about by aging and fermentation	Wyndmoor, Pa.	Yes	8-C-1
E5 3-30(C)**	Carcinogenic and cocarcinogenic activities of various tobaccos, smoke condensates, and related substances by biological assay	Lexington, Ky.	No	
E5 3-31(C)**	Effects of combinations of cigarette smoke and nononcogenic viruses on the development of lung tumors in rodents	Lexington, Ky.	No	
E5 3-32(C)**	Response of virus-cell culture systems to tobacco smoke condensate and fractions	Lexington, Ky.	No	

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E5 3-33 (C)**	Effect of tobacco smoke constituents on rodent tracheal rings grown in tissue culture	Lexington, Ky.	No	
E5 3-34 (C)**	Response of selected oral anaerobes to the saliva-thiocyanate system of smokers and on the thiocyanate and Polonium <sup>210</sup> activity of calculi from smokers and non-smokers	Lexington, Ky.	No	
E5 3-35 (C)**	Improved analytical method for determining polynuclear aromatic hydrocarbons and heterocyclic compounds in cigarette smoke	Lexington, Ky.	No	
E5 3-36 (C)**	Isolation and identification of the higher boiling heterocyclic nitrogen compounds in cigarette smoke	Lexington, Ky.	No	
E5 3-37 (C)**	Effect of structural variations on the composition of amino acid pyrolysates	Lexington, Ky.	No	
E5 3-38 (C)**	Effect of aqueous extracts of cigarette smoke condensate on tumorigenesis in rats	Lexington, Ky.	No	
E5 3-39 (C)**	Influence of combustion modifiers on the vapor phase constituents of cigarette smoke	Marcus Hook, Pa.	No	
E5 3-40 (C)**	Effect of tobacco smoke condensate on oral carcinogenesis	Lexington, Ky.	No	

\* Discontinued during reporting year.

\*\* Initiated during reporting year.

LINE PROJECT CHECK LIST -- REPORTING YEAR 66 to 67

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area and Subheading
E5 1	Sugar and Sirups Investigations.			
E5 1-76	Fermentation studies on maple	Wyndmoor, Pa.	Yes	9-B-1
E5 1-78(C)	Maple sap storage	Bainbridge, N. Y.	Yes	9-C-1
E5 1-82	Sap concentration by reverse osmosis	Wyndmoor, Pa.	Yes	9-C-2
E5 1-84**	Maple flavor composition	Wyndmoor, Pa.	Yes	9-A-1

\*\* Initiated during reporting year.







